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MspI, an isoschizomer of HpaII which cleaves both unmethylated and methylated HpaII sites

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ABSTRACT

The cleavage of DNA by restriction endonucleases HpaII and HapII is prevented by the presence of a 5-methyl group at the internal C residue of its recognition sequence CCGG. MspI, an isoschizomer of HpaII available from New England Biolabs, cleaves DNA irrespective of the presence of a methyl group at this position. This enzyme cleaves DNA from Haemophilus parainfluenzae and Haemophilus aphrophilus readily while HpaII and HapII cannot degrade these DNAs. Practically all HpaII sites in mammalian sperm DNA are also protected by methylation at the internal C position since HpaII and HapII barely cleave this DNA (average molecular weight 40 kb). MspI, however, cleaves the DNA to an average size of about 5 kb.

INTRODUCTION

The possession of a restriction endonuclease by a bacterium requires that the bacterium protects its own chromosomal DNA from degradation by the endogenous restriction endonuclease. This protection is afforded by the modification of the cleavage sites recognised by the restriction endonuclease [1]. Specific modification of these sites by methylation of adenines at the 6 position (e.g. the EcoRI system modifies GAATTC → GA^{m}ATTC) or cytosines at the 5 position (in H. parainfluenzae the HpaII sites - CCGG - are probably methylated at the internal C residue of the site [2]) have been described.

Methylation of DNA occurs in higher organisms, but seems in this case to be restricted to 5-methyl C residues present in the doublet 5'-CG-3' [3]. Bird and Southern [4] recently described an ingenious use of the bacterial restriction modification system to study DNA methylation in higher eukaryotes. They showed that many restriction endonucleases with recognition sites containing the sequence -CG- (e.g. HpaII, CCGG; HhaI, GCGC) are essentially incapable of cleaving at their respective recognition sites (with

a few important exceptions) in Xenopus ribosomal DNA isolated from blood DNA because of the presence of a 5-methyl group at the internal C residues of these sites. In a study of the methylation of HpaII sites close to the rabbit β-globin gene we noted that MspI, an isoschizomer of HpaII, readily cleaves mammalian DNA. We show here that this enzyme is capable of cleaving DNA at CCGG sites irrespective of the presence of a 5-methyl group at the internal C residue. This enzyme, used in conjunction with HpaII, should be valuable for the study of DNA methylation in higher organisms.

MATERIALS AND METHODS

Bacterial DNAs were prepared as described for rabbit DNAs [5] except that lysis was achieved by lysozyme treatment (250 µg/ml for 5 min at 0°C) followed by the addition of sodium dodecylsulphate to a final concentration of 1% and incubation at 60°C for 5 min. Rabbit sperm DNA was prepared as described [5].

Digestion of DNAs with restriction endonucleases was done in 10 mM Tris-HCl (pH 7.5), 8 mM $MgCl_2$, 1 mM dithiothreitol and 0.01% autoclaved gelatin at 37°C for 2-4 h. Analysis of digests by agarose gel electrophoresis was done as described [5]. MspI was purchased from New England Biolabs. Details regarding this enzyme can be obtained from them.

RESULTS AND DISCUSSION

Digestion of rabbit DNA with endonucleases HpaII and HapII generates larger DNA fragments than would be expected on the basis of the frequency of the CpG doublet in this DNA [6]: by analogy with the Xenopus ribosomal DNA system described by Bird and Southern [4] and the known levels of 5-methyl cytosine in mammalian DNA, we infer that the majority of HpaII sites is methylated in rabbit DNA. To our surprise we observed that MspI gave small DNA fragments (Fig. 1). The differences observed were unlikely to be due to trivial factors, since phage lambda DNA added to an aliquot of each of the three digests displayed in Fig. 1 was digested to the characteristic HpaII limit digest pattern. Moreover, the differences in the MspI and HapII digests of rabbit DNA are not due to differences in the tetranucleotide recognized, or to a contaminating enzyme in the MspI, because identical fragmentation patterns for both enzymes were found

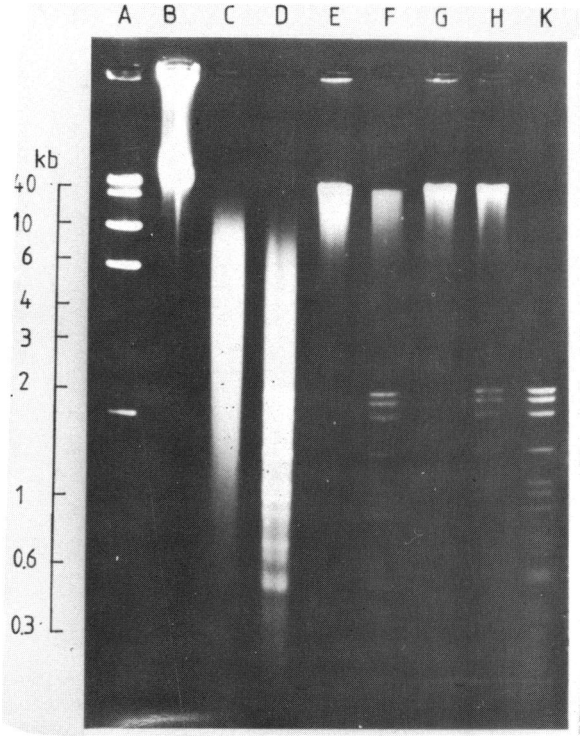

Fig. 1. Digestion of rabbit sperm DNA with MspI, HapII and HpaII. Rabbit DNA was digested with the respective restriction endonucleases and analysed by electrophoresis in a 0.8% agarose gel. A: A mixture of marker phage lambda DNA (48 kb), phage Ø29 DNA (17 kb) and phage Ø29 DNA x EcoRI (9.2, 5.4, 1.6, 0.8, 0.54 kb). B: 0.12 µg rabbit sperm DNA input. C: 0.5 µg rabbit sperm DNA x MspI. D: as C, but with 0.5 µg phage lambda DNA added as internal control. E: 0.12 µg rabbit sperm DNA x HapII. F: as E, but with 0.5 µg phage lambda DNA added. G: 0.12 µg rabbit sperm DNA x HpaII. H: as G, but with 0.5 µg of phage lambda DNA added. K: 0.5 µg phage lambda DNA x HapII.

with a series of phage DNAs and with plasmid pCR1, as shown in Fig. 2.

A simple explanation for the difference between these isoschizomers is that MspI cleaves DNA at CCGG sites, irrespective of the presence of a 5-methyl group at the internal C residue, whereas the HpaII and HapII enzymes cleave only those CCGG sites that are unmethylated at this position. To test this we examined the ability of the three isoschizomers to cleave DNAs protected against HpaII or HapII cleavage by <u>in vivo</u> methylation. Mann and Smith [2] have shown that HpaII cannot cleave the sequence $-C^{m}CGG-$ whereas the sequence $-^{m}CCGG-$ is cleaved. The methylation

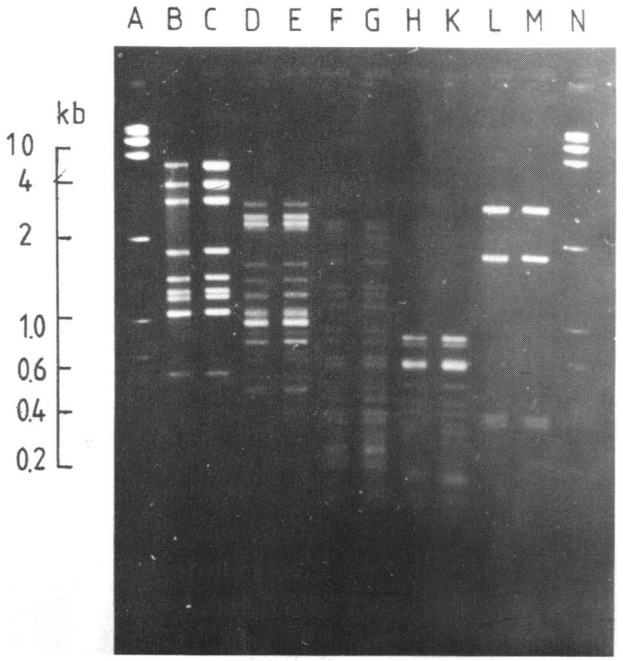

Fig. 2. Digestion of various viral DNAs and plasmid pCR1 DNA with endonucleases HapII and MspI. The DNAs were digested with the respective restriction endonucleases and analysed by electrophoresis in a 1.2% agarose gel. A: Marker DNAs specified in Fig. 1. B: Phage Ø29 DNA x HapII. C: Phage Ø29 DNA x MspI. D: Phage T_7 DNA x HapII. E: Phage T_7 DNA x MspI. F: Phage lambda DNA x HapII. G: Phage lambda DNA x MspI. H: Plasmid pCR1 DNA x HapII. K: Plasmid pCR1 DNA x MspI. L: Phage ØX174 (replicative form) x HapII. M: Phage ØX174 (replicative form) x MspI. N: as A.

of the internal C residue presumably occurs in vivo in H. parainfluenzae. If the modification system is the same in both H. parainfluenzae and H. aphrophilus, we should expect that HpaII and HapII can cleave neither H. parainfluenzae nor H. aphrophilus DNAs. Fig. 3 shows that this is the case. MspI, however, cleaves both H. aphrophilus and H. parainfluenzae DNA. We conclude that MspI can cleave $C^{m}CGG$ sites.

There are two practical implications of this finding. First, caution should be exercised in the use of isoschizomers in the place of well-characterized restriction endonucleases in the analysis of DNA where methylated C or A residues may be present. This is especially important where mammalian DNAs, containing $-^{m}CpG-$ sequences, are analysed with restriction endonucleases with a recognition site containing a -CpG- sequence. Second,

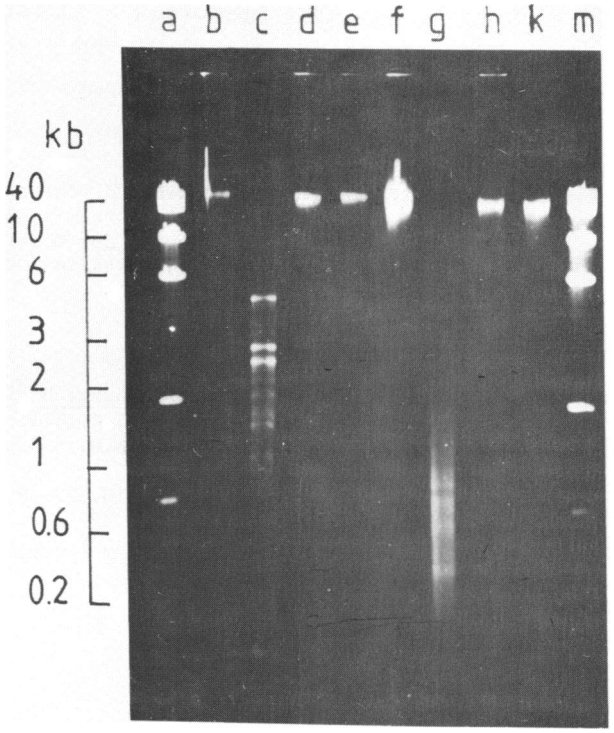

Fig. 3. Digestion of Haemophilus parainfluenzae and H. aphrophilus DNAs with MspI, HapII and HpaII. The bacterial DNAs were digested with the respective restriction endonucleases and analysed by electrophoresis in a 0.8% agarose gel. A: Marker DNAs specified in Fig. 1. B: 0.12 µg H. parainfluenzae input. C: 1 µg H. parainfluenzae DNA x MspI. D: 0.12 µg H. parainfluenzae DNA x HapII. E: 0.15 µg H. parainfluenzae DNA x HpaII. F: 0.3 µg H. aphrophilus DNA. G: 1 µg H. aphrophilus DNA x MspI. H: 0.15 µg H. aphrophilus DNA x HapII. K: 0.15 µg H. aphrophilus DNA x HpaII. M: Markers as in Fig. 1. The strong bands in lane C are derived from plasmid DNA present in H. parainfluenzae. All digestions were complete as judged by digestion of an internal marker of phage lambda DNA added to an aliquot of the digest.

commercial suppliers of restriction endonucleases should ensure that the enzymes sold are clearly described. In this case, New England Biolabs advertised HpaII in their catalogue, yet the enzyme shipped was in fact the isoschizomer described above and described on the data sheet as isoschizomer-HpaII (this has now been corrected).

The availability of isoschizomer pairs with methylation-sensitive and methylation-insensitive enzymes makes it possible to assay for DNA methylation at the cleavage sites for these enzymes in any DNA. Using these enzymes in conjunction with a specific

hybridization system [5] has made it possible to analyse a single under-methylated CCGG site in the region of the rabbit β-globin gene. This will be the subject of another report.

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REFERENCES

1 Meselson, M., Yuan, R. and Heywood, J. (1972) Ann.Rev.Biochem. 41, 447-466.
2 Mann, M.B. and Smith, H.O. (1977) Nucl.Acids Res. 4, 4211-4221.
3 Vanyushin, B.F., Tkacheva, S.G. and Belozersky, A.N. (1970) Nature 225, 948-949.
4 Bird, A. and Southern, E.M. (1978) J.Mol.Biol. 118, 27-48.
5 Jeffreys, A.J. and Flavell, R.A. (1977) Cell 12, 429-439.
6 Swartz, M.N., Trautner, T.A. and Kornberg, A. (1962) J.Biol.Chem. 237, 1961-1967.