DNA methyltransferases affecting the sequence 5'CCGG

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ABSTRACT

B. subtilis phage SPß and Moraxella sp. code for DNA methyl-
transferases which methylate both cytosines of the sequence
5'CCGG. Experiments using a B. subtilis strain whose DNA is
sensitive to HpaII and resistant to MspI degradation, indicated
that methylation of the outer C of this sequence provides pro-
tection against the restriction enzyme MspI.

INTRODUCTION

The dinucleotide sequence CpG is found with exceptionally low
incidence in the DNA of eukaryotes (1). Methylation of eukary-
otic DNA occurs prevalently on the C contained in this rare
sequence (2, 3, 4). Recently results have accumulated in
various laboratories indicating that such C-methylation is in-
volved in the regulation of transcription of the genetic message
in eukaryotes (5, 6, 7, 8, 9). Portions of such DNA which are
actively transcribed show very little methylation in this
sequence, whereas CpG in the same regions is highly methylated
when transcriptionally dormant. This observation has led to an
increased interest in the methylation of DNA and analysis of the
substrate character of DNA for degradation by restriction enzymes
or modifiability through modification enzymes. Such studies per-
mit precise information on the specificity and extent of methyl-
ation, provided that the restriction/modification specificities
of the enzymes are known. Of particular interest here are
enzymes which contain the dinucleotide CpG in their target
sequence, e.g. HhaI (GCGC), HpaII (CCGG), or MspI (CCGG). The
HhaI- and HpaII-specific modifications have been shown to be
mediated through methylation of the central C in the respective

target sequences (10, 11). Sneider (12), van der Ploeg et al.
(13) and this laboratory (14) have suggested that methylation
of C at the 5' end of the MspI target sequence was responsible
for modification. However, a direct determination of MspI modi-
fication was still lacking. Here we report experiments which
provide such direct proof. We also demonstrate that B. subtilis
phage SPß and Moraxella sp. code for enzymes which methylate
both adjacent C's in the target sequence CCGG.

MATERIALS AND METHODS

  The bacteria used in this study are compiled in Table 1.
Strains of B. subtilis and Moraxella sp. were grown in TY medium
(15). The SPß19 lysogenic strain TB104 was induced with mitomy-
cin as described (16). Bacterial DNA was extracted as described
by Marmur (17). H. parainfluenzae DNA was a gift of Dr. R.
Gramkova. Phage SPß DNA was prepared from purified phage as
described for SPP1 (18). Restriction enzymes MspI, HpaII and
HaeIII were purchased from Biolabs (Beverly, Ma., USA) and
Boehringer (Mannheim, Germany) and used as described by the
manufacturers. Analytical agarose gel electrophoresis was per-
formed as described (19). Localization of the methylated nucleo-
tide in the sequence CCGG was performed by the method described
by Günthert et al. (20). Base sequences are always written in
the direction 5'→3'; $\overset{*}{C}$ stands for 5-methyl-cytidine-monophos-
phate.

TABLE 1
BACTERIAL STRAINS USED

STRAIN	MODIFYING ACTIVITY AFFECTING GGCC OR CCGG	COMMENTS/ORIGIN
B. subtilis SB1207 (SPß)⁻	none	(16)
B. subtilis TB804 (SPß)⁻	GG$\overset{*}{C}$C	(16)
B. subtilis TB104 (SPß19)⁺	$\overset{**}{C}$CGG	Modifying activity manifested only after induction of pro-phage SPß19 (21)
B. subtilis Q	$\overset{*}{C}$CGG	Strain was originally obtained from B. Delaporte. The strain is not related to B. subtilis 168 strains.
Moraxella sp.	$\overset{**}{C}$CGG	Strain obtained from R. J. Roberts. Original source of MspI restriction enzyme.

RESULTS

   B. subtilis phage SPß and Moraxella sp. code for methyltrans-
ferases which cause resistance of DNA to both MspI and HpaII
degradation. B. subtilis phage SPß codes for a methyltransfe-
rase which methylates the internal C of the BsuR recognition
site GGCC (16). The gene of SPß responsible for this modifying
activity was identified through the discovery of SPß mutants
which had lost the capacity for such methlyation. SPß also
codes for modifying activities which affect MspI and HpaII re-
striction. Independent BsuR methyltransferase deficient mutants
were isolated and found to fall into two classes depending on
whether their DNA was sensitive to degradation by MspI and HpaII.
The degradation patterns with restriction enzymes MspI, HpaII and
HaeIII (an isoschizomer of BsuR) of DNA derived from SPß phages
is shown in Figure 1. SPß WT DNA was resistant, while SPß26 DNA
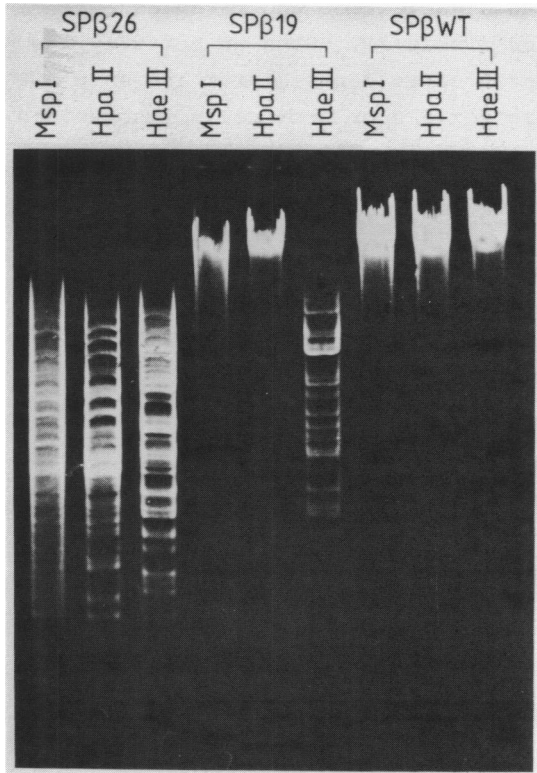

Figure 1. Gel electro-
phoresis of SPß DNAs
after degradation with
restriction enzymes
MspI, HpaII and HaeIII.

was sensitive to all enzymes tested. DNA from the BsuR sensitive mutant SPβ19 was resistant to both MspI and HpaII digestion.

Neither phage crosses nor mutagenesis resulted in SPβ mutants affected in either MspI or HpaII modification alone. This suggested that the simultaneous presence or absence of both modifications was caused by one and the same enzyme activity. Protection against HpaII is mediated by methylation of the central C of the MspI/HpaII target sequence CCGG (11), but this methylation does not produce MspI modification. It therefore seemed probable that MspI modification involved the outer C of the target sequence. The presence of a methyltransferase affecting this nucleotide was readily demonstrable in cell-free extracts of the induced strain TB104 which is lysogenic for SPβ19. Such an extract was employed to methylate BsuR modified TB804 DNA in vitro, using Ado|Me-^{3}H|Met as the methyl donor. The subsequent determination of the distribution of radioactivity between the two C residues of the isolated dinucleotide CpC revealed that both C's had become methylated (Table 2). Such an activity was not demonstrable in cell-free extracts derived from isogenic cells which were not lysogenic for SPβ or from cells lysogenized with the MspI/HpaII restrictable phage SPβ26 (data not shown).

The methylation pattern with SPβ19 is analogous to that described for DNA isolated from Moraxella sp.. Moraxella sp. DNA is also resistant to both MspI and HpaII (J. Brooks and R. Roberts, pers. commun.), but sensitive to HaeIII (Fig. 2). Here, too, a cell free extract of Moraxella sp. will methylate either C residue in the sequence CCGG (Table 2).

Methylation of the outer C in the sequence 5'CCGG is sufficient to cause resistance of DNA to MspI degradation. Our data suggested that MspI-modification in the sequence 5'CCGG is either due to methylation of both C's or to methylation of the outer C alone. DNA from strain B. subtilis Q was found to be resistant to MspI but sensitive to HpaII (Figure 2). A cell free extract from this strain only methylated the outer C of the MspI/HpaII target sequence (Table 2). Thus methylation of this C is sufficient to produce protection against MspI restriction.
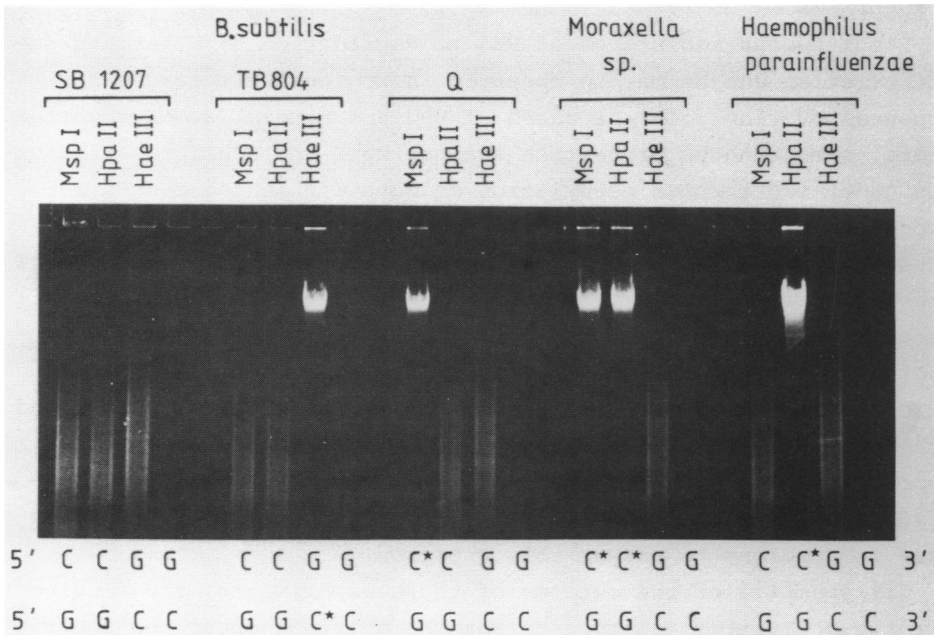
Figure 2. Gel electrophoresis of MspI, HpaII and HaeIII restricted bacterial DNAs. The methylated site(s) of the sequences CCGG and GGCC are indicated below.

TABLE 2

LOCALIZATION OF THE ^{3}H-LABELLED METHYLGROUP IN d(CpC).

CELL FREE EXTRACT OF	PHOSPHO-DIESTERASES[1]	DISTRIBUTION OF RADIOACTIVITY BETWEEN (%)			TYPE OF MODIFICATION
		dC	d(CpC)	dpC/dCp	
B. subtilis TB1O4 (SPβ19)[+]	SV	46.4	6.9	46.7	** CCGG
	CS	47.8	4.0	48.2	
Moraxella sp.	SV	49.8	1.0	49.2	** CCGG
	CS	48.0	2.7	49.3	
B. subtilis Q	SV	96.3	1.4	2.3	* CCGG
	CS	9.7	13.0[2]	77.3	

[1]SV = Snake Venom P'diesterase, CS = Calf Spleen P'diesterase

[2]Possibly due to incomplete digestion with calf spleen P'diesterrase

DISCUSSION

Simultaneous resistance of DNA to degradation by more than one restriction enzyme may be observed in regions were target sequences overlap. In the cases of SPß and Morexella DNA reported here, simultaneous protection against MspI and HpaII restriction is generated through methylation of both C's in the common recognition sequence 5'CCGG. Single methylation of the inner C generates HpaII resistance (11) and of the outer C generates MspI resistance (Table 2). The methyltransferase with the latter specificity was identified in B. subtilis Q and is currently being purified. We are also searching for a matching restriction enzyme which would be an isoschizomer of MspI. Are one and the same or two distinct enzymes responsible for simultaneous protection against MspI and HpaII? Purification of the enzymatic activities from Moraxella sp. and SPß lysogenic B. subtilis are in progress to answer this question.

Irrespective of the outcome of these experiments, the finding of the activities observed raises the question about the function of such methyltransferases. To our knowledge, no restricting activity with HpaII specificity was observed in Moraxella sp.. Neither do we know of any potential host cell for SPß which have MspI/HpaII restriction activity. As more methyltransferases are identified, which exist in the absence of matching restriction enzymes, it becomes difficult to rationalize the presence of such methyltransferases simply as enzymes which serve to protect DNA against restriction.

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