

GENE 988

Correlation of DNA adenine methylase activity with spontaneous mutability in *Escherichia coli* K-12[†]

(Recombinant DNA; *tac* promoter; multicopy plasmid; mismatch repair)

M.G. Marinus, Anthony Poteete* and Judy A. Arraj

Department of Pharmacology and *Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01605 (U.S.A.) Tel. (617) 856-3330

(Received November 13th, 1983)

(Revision received and accepted January 17th, 1984)

SUMMARY

Using a multicopy plasmid in which the *tac* promoter has been placed in front of the *dam* gene of *Escherichia coli* K-12, we show that levels of DNA adenine methylase activity are correlated with the spontaneous mutation frequency.

INTRODUCTION

Wagner and Meselson (1976) suggested that mismatch repair in *E. coli* may correct potential mutations that arise as replication errors in daughter DNA strands. Since newly synthesized DNA strands are undermethylated (Marinus, 1976), DNA methylation might allow the repair process to distinguish between old and new strands. Support for this idea has been obtained using differentially methylated mismatched bacteriophage heteroduplexes,

both in vivo (Pukkila et al., 1983) and in vitro (Lu et al., 1983). The unmethylated strand in such heteroduplexes is repaired preferentially and fully methylated heteroduplexes are resistant to correction.

One line of evidence that such a post-replicative system operates in *E. coli* comes from the experiments of Herman and Modrich (1981). A plasmid (pGG503) which overproduces the *dam* methylase 20- to 50-fold was found to confer hypermutability on strains carrying it. Such hypermutability would be expected if methylation occurs on daughter DNA strands before mismatch repair, since fully methylated DNA is not repairable. Other plasmids containing the *dam* gene, pLC13-42 (Clarke and Carbon, 1976), *pdam118* (Brooks et al., 1983) and pMQ3 (Arraj and Marinus, 1983), do not confer hypermutability (not shown), presumably because they overproduce the methylase only two-fold (*pdam118*) or ten-fold (pLC13-42, pMQ3).

[†] Dedicated to the memory of Ahmad I. Bukhari.

Abbreviations: *bla*, β -lactamase gene; bp, base pairs; *dam*, gene encoding an enzyme which methylates -GATC- sequences in DNA (Marinus, 1984); IPTG, isopropylthio- β -galactopyranoside; 6-meA, 6-methyladenine; Nal^r, naladixic acid resistance; p, plasmid; Rif^r, rifampicin resistance; Str^r, streptomycin resistance; *tac*, hybrid *trp-lac* promoter (De Boer et al., 1983).

EXPERIMENTAL AND DISCUSSION

(a) Plasmid construction and methylase assay

We wanted to test the relationship between methylase activity and mutability in a system in which *dam* was the only cloned *E. coli* gene, and in which the amount of methylase could be varied. To this end, we constructed pTP166, a multicopy plasmid derived from pBR322 (Fig. 1). In this plasmid the *tac* promoter (De Boer et al., 1983) is 5' to a 1140-bp fragment of *E. coli* DNA containing the 854-bp *dam* gene (Brooks et al., 1983). This plasmid was transformed into *E. coli* W3110 *lacI*^qL8 *dam*⁺ (Brent and Ptashne, 1981), in which the *lacI* product represses transcription from the *tac* promoter. Table I shows that there is a marked increase in spontaneous mutability and methylase production after IPTG is added to cells carrying pTP166. No difference in mutability is seen between plasmidless cells, those carrying a plasmid (pMQ143) with a deleted *tac* promoter, or those bearing a plasmid (pMQ167) in which the amino-terminal end of the *dam* gene has been deleted (Table I). The slight increase in mutability in cells carrying pTP166 in the absence of inducer could be

TABLE I

DNA adenine methylation and spontaneous mutability in *E. coli*

The host strain, W3110, carries the *lacI*^q and *lacPL8* mutations and is *dam*⁺. pMQ143, which lacks the *tac* promoter, was constructed by digesting pTP166 with *EcoRI* and *XbaI*, filling in the ends with deoxyribonucleoside triphosphates using the large fragment of DNA polymerase I, and transforming into a *lac*⁺ strain. In pMQ167, the *BamHI* fragment containing the amino-terminal end of the *dam* gene has been deleted (see Fig. 1). To determine mutation frequency, a few hundred cells were inoculated into Difco Brain Heart Infusion broth (20 g/l), with or without 0.5 mM IPTG and grown to saturation. Portions were spread on Brain Heart Infusion agar containing 100 µg/ml of antibiotic and incubated at 37°C for 24–48 h. Mutation frequency is expressed as the number of resistant mutants per 10⁸ cells plated. At least five independent cultures were used for each strain. Methylase activity was measured by growing cells in 100 ml Brain Heart Infusion broth to 1–2 × 10⁸/ml, adding IPTG to 1 mM if required, and incubating for an additional 2 h. The cells were harvested, sonicated and centrifuged, and enzyme activity in the extract was measured as described by Bale et al. (1979).

Plasmid	P _{tac}	<i>dam</i> ^a	IPTG	Mutation frequency (per 10 ⁸)			Relative methylase activity
				Rif ^r	Nal ^r	Str ^r	
–	–	–	–	0.6	0.1	0.04	–
	–	–	+	0.6	0.3	0.04	1
pMQ143	–	+	–	0.9	0.3	–	–
	–	+	+	0.6	0.3	–	15
pMQ167	+	–	–	1.4	0.2	–	–
	+	–	+	2.3	0.6	–	1
pTP166	+	+	–	3.4	0.8	0.08	11
	+	+	+	14.4	12.2	1.34	300–500

^a Refers to the *dam* gene on the plasmid.

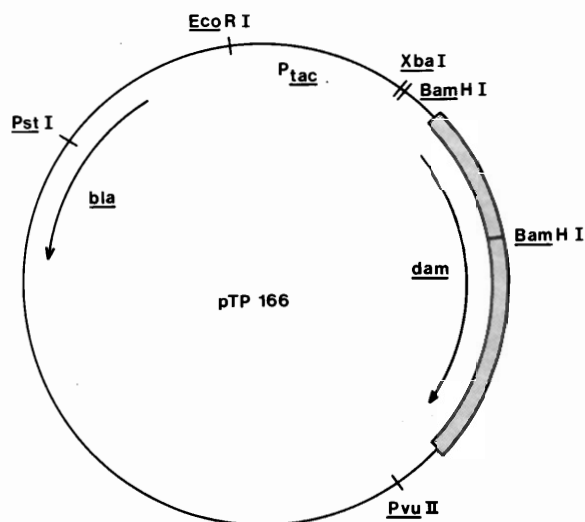


Fig. 1. Physical map of the pTP166 recombinant plasmid. The *PstI*–*XbaI* fragment, including the P_{tac} promoter, was obtained from *ptac12* (a gift from E. Amman and J. Brosius), and the remainder of pTP166 is derived from *pdam118* (Brooks et al., 1983). The stippled box indicates the *dam* gene. Arrows specify the orientation of transcription.

due to the presence in the population of a small fraction of plasmids that have mutated to constitutivity. Using the values of the controls as unity, in-

duced pTP166-containing cells show 18-, 41- and 33-fold increases in mutability to rifampicin, naladixic acid and streptomycin resistances, respectively.

(b) Mutability and mismatch repair

The data are compatible with the results and models described by Herman and Modrich (1981), Pukhila et al. (1983) and Lu et al. (1983). In uninduced cells carrying pTP166, post-replicative mismatch repair would correct the lesion in the new DNA strand prior to DNA methylation. In induced cells, the new DNA strand would be methylated prior to repair, and base-pair mismatches would not be corrected and lead subsequently to mutation.

Hypermutability could also result from random repair of mismatches in fully methylated duplex DNA molecules. In this case repair would occur half of the time in the old DNA strand and half in the new. Repair of the old strand would lead to mutation. Random repair is thought to occur in *dam* mutants and to be responsible for various phenotypic traits of such mutant strains (Marinus, 1984). The presence of pTP166, however, suppresses those *dam*⁻ phenotypes that have been tested (not shown), indicating that repair does not occur in a random manner in cells containing *dam*⁺ plasmids.

Another explanation for the hypermutability would be that at high enzyme concentrations the *dam* methylase loses specificity and methylates sequences in addition to -GATC-. This should lead to an increase in the mol % of 6-meA in DNA. No difference in 6-meA content was found between induced and uninduced pTP166-containing cells (not shown) using the method described by Bale et al. (1979). Since there is an experimental error of 5% in these determinations, however, an effect of low-level methylation at sequences other than -GATC- cannot be excluded.

(c) Applications

Strains carrying pTP166 should be useful for several other purposes. These include serving as: (i) a source of enzyme for purification of the *dam* product; (ii) serving as a host for the growth of certain bacteriophages (e.g., lambda) whose DNA is incompletely methylated in normal *E. coli* strains; and (iii) for in vitro mutagenesis studies in which artifi-

cially constructed heteroduplexes containing a mismatched base pair are used. Such heteroduplexes can be either methylated in vitro using enzyme prepared from pTP166-containing cells or methylated in vivo by transforming into pTP166-containing hosts. In both cases mismatch repair should be prevented, giving a 1 : 1 mixture of each parental type.

ACKNOWLEDGEMENTS

This work was supported by USPHS grant GM30330. We thank Alexis Z. Frey and Margaretha Carraway for technical assistance, and Joan Brooks, E. Amman and J. Brosius for gifts of plasmids.

REFERENCES

- Arraj, J.A. and Marinus, M.G.: Phenotypic reversal in *dam* mutants of *Escherichia coli* K-12 by a recombinant plasmid containing the *dam*⁺ gene. *J. Bacteriol.* 153 (1983) 562-565.
- Bale, A., D'Alarcoa, M. and Marinus, M.G.: Characterization of DNA adenine methylation mutants of *Escherichia coli* K-12. *Mutation Res.* 59 (1979) 157-165.
- Brent, R. and Ptashne, M.: Mechanism of action of the *lexA* gene product. *Proc. Natl. Acad. Sci. USA* 78 (1981) 4204-4208.
- Brooks, J.E., Blumenthal, R.M. and Gingeras, T.R.: The isolation and characterization of *Escherichia coli* DNA adenine methylase (*dam*) gene. *Nucl. Acids Res.* 11 (1983) 851-871.
- Clarke, L. and Carbon, J.: A colony bank containing synthetic ColE1 hybrid plasmids representative of the entire *E. coli* genome. *Cell* 9 (1976) 91-99.
- De Boer, M.A., Comstock, L.J. and Vasser, M.: The *tac* promoter: a functional hybrid derived from the *trp* and *lac* promoters. *Proc. Natl. Acad. Sci. USA* 80 (1983) 21-25.
- Herman, G.E. and Modrich, P.: *Escherichia coli* K-12 clones that overproduce *dam* methylase are hypermutable. *J. Bacteriol.* 145 (1981) 644-646.
- Lu, A.-L., Clark, S. and Modrich, P.: Methyl-directed repair of DNA base pair mismatches in vitro. *Proc. Natl. Acad. Sci. USA* 80 (1983) 4639-4643.
- Marinus, M.G.: Adenine methylation of Okazaki fragments in *Escherichia coli*. *J. Bacteriol.* 182 (1976) 853-854.
- Marinus, M.G.: Methylation of prokaryotic DNA, in Razin, A. Cedar, H. and Riggs, A. (Eds.), *DNA Methylation and its Biological Significance*. Springer-Verlag, New York, 1984, in press.
- Pukhila, P., Peterson, J., Herman, G., Modrich, P. and Meselson, M.: Effects of high levels of DNA adenine methylation on methyl-directed mismatch repair in *E. coli*. *Genetics* 104 (1983) 571-582.
- Wagner, R.W. and Meselson, M.: Repair tracts in mismatched DNA heteroduplexes. *Proc. Natl. Acad. Sci. USA* 73 (1976) 4135-4139.