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Expression of a DNA Methylation (*dam*) Gene in *Escherichia coli* K-12

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Abstract. Plasmid pMQ3, carrying the *dam* gene of *Escherichia coli* on a 6.1 Kb fragment, shows a tenfold increase in relative DNA adenine methylase activity, while plasmid p*dam*118, with a 1.14 Kb *dam* insert, shows only a twofold increase, although both plasmids were derived from plasmid pLC13-42. Since a copy number effect did not seem to be the cause of this difference, we have subcloned pMQ3 in order to determine whether the additional chromosomal DNA present in this plasmid is responsible for the enhancement of methylase activity. We show that the 346 base pairs upstream of *dam* contain sequences necessary for expression. DNA sequence analysis has revealed that in p*dam*118 only the 118 bases 5-prime to the *dam* gene are present in other constructs and that the additional upstream material is pBR322 DNA. This shows that p*dam*118 carries a DNA duplication.

The product of the *dam* gene of *Escherichia coli* K-12 is a DNA adenine methylase which methylates —GATC— sequences in newly synthesized DNA at or near the replication fork [14, 15]. Although the *dam* gene product is not essential for cell viability [13, 19], the pleiotropic phenotype of *dam* mutant strains [14] suggests that either DNA adenine methylation or the methylase may have a role in several cellular functions [14, 15]. Recent evidence suggests that *dam* methylation is involved in (i) mismatch repair [12, 20], (ii) control of transcription of certain genes that have one or more —GATC— in promoter sequences [15], and (iii) initiation of DNA replication at *ori*C, since plasmids containing *ori*C do not replicate in *dam* mutants [10, 16, 22]. There is little information, however, on whether or how the *dam* gene is regulated.

Plasmid pLC13-42, from the Clarke and Carbon colony bank, is a large ColE1 hybrid plasmid which carries both the *dam* and *trpS* genes [7, 9]. Herman and Modrich [9] subcloned the *dam* and *trpS* genes from pLC13-42 on a large *Pst*I fragment of 23 Kb into plasmid pBR322 to give plasmid pGG503. The *dam* gene on pGG503 was further subcloned to give two additional plasmids, pMQ3 [2], containing 6.1 Kb and p*dam*118 [4], containing 1.14 Kb of insert DNA.

A *dam* strain carrying plasmid pMQ3 shows a relative methylase activity approximately tenfold over that of a dam^+ strain [2]. However, we have found that p*dam*118 contains only a one- to threefold increase in relative methylase activity. Since pMQ3 contains more chromosomal DNA than does p*dam*118, we have undertaken an analysis of pMQ3 in order to determine whether any of the additional chromosomal DNA present in pMQ3 is responsible for the apparent enhancement of methylase activity.

Materials and Methods

Chemicals and media. 2-Aminopurine (2-AP), chloramphenical (Cam), and ampicillin (Amp) were obtained from Sigma (St. Louis, Missouri). Restriction endonucleases were obtained from New England Biolabs (Beverly, Massachusetts) or Boehringer-Mannheim Corp. (Indianapolis, Indiana), and reaction conditions were those specified by the supplier. Complete and minimal media were as previously described [3]. Amp or Cam was added to media at a final concentration of 40 μg/ml, respectively. 2-AP was added to plates at 100 μg/ml.

Enzyme assays. β-Galactosidase assays were performed in whole cells by the method of Miller [17]. β-Lactamase activity was determined by the method of O'Callaghan et al. [18] with a chromogenic cephalosporin substrate, Nitrocefin (Glaxo Laboratories, Greenford, U.K.). Methylase assays were done by the method of Bale et al. [3].

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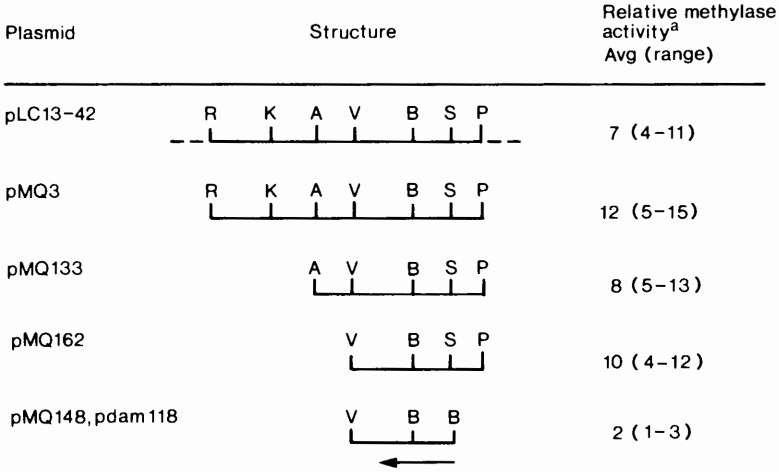

Fig. 1. Structure and relative methylase activities of plasmid constructs containing the *dam* gene. All plasmids are derived from pLC13-42, which contains *Escherichia coli* K-12 chromosomal DNA, including the *dam* gene. Only chromosomal DNA is shown in this figure. R, K, A, V, B, S, and P denote *Eco*R1, *Kpn*1, *Sal*I, *Pvu*II, *Bam*H1, *Sau*3A, and *Pst*I sites, respectively. The open reading frame for the *dam* gene is shown by an arrow. The plasmids were introduced into GM33 (*dam-3*), and the methylase activity was determined as described in Materials and Methods.

[a] Methylase activity is expressed as relative to that in the *dam*$^+$ strain, GM28, from which GM33 was derived.

Bacterial strains and plasmids. *Escherichia coli* strains GM33 (*dam-3*), GM1674 [F-*lacI*^{q} del M15 *pro*$^+$/del(*lac-pro*) *dam-3 dcm-6 tsx-78 glnV44 relA1*], GM1684 [F-*lacI*^{q} del M15 *pro*$^+$/del(*lac-pro*) *dam-4 thi-1 glnV44*], and GM1855 [del(*lac-pro*) *dam-4 thi-1 glnV44*] were used. Plasmids pLC13-42 [7], p*dam*118 [4], and pMQ3 [2] have been described previously. *Plasmid pMQ133*: a 6.8 Kb plasmid containing the larger *Sal*I-*Pst*I fragment of pBR322, a 1.9 Kb *Pst*I fragment carrying the *cat* gene from Tn*9* [1] and a 1.8 Kb *Pst*I-*Sal*I fragment carrying the *dam* gene. *Plasmid pMQ162*: The 1.24 Kb *Pst*I-*Pvu*II (Fig. 1) fragment of pMQ133 was subcloned into plasmid pMZ152 [23]. *Plasmid pMQ148*: Plasmid p*dam*118 was digested with *Eco*RV and *Pvu*II and the 1.33 Kb fragment ligated to the larger *Eco*RV and *Pvu*II fragment of pBR322. Plasmids pMQ148 and p*dam*118 carry the same insert as shown in Fig. 1. *Plasmids pMQ132, pMQ139,* and *pMQ159*: Plasmid pMC931 contains a 6.8 Kb *Bam*HI fragment (*lacZ*$^-$ *lacY*$^+$) in which the first eight codons of the amino-terminal end of the *lacZ* gene were removed [6]. The *lac* genes will be trascribed only when an active promoter is upstream. We inserted the 6.8 Kb *lac* fragment into the *Bam*HI site within the *dam* gene of plasmids p*dam*118, pMQ133, and pMQ148, to yield plasmids pMQ132, pMQ139, and pMQ159 respectively. *Plasmid pMQ163*: The 0.6 Kb *Pst*1-*Bam*H1 fragment of pMQ133 was subcloned into pEMBL8 [8] by selection for Lac$^-$ colonies after transformation.

DNA sequencing. Single-stranded plasmid DNA was obtained by infecting pMQ163 containing cells with phase IR1 [8] and sequenced according to the method of Sanger et al. [21]. Double-stranded sequencing of pMQ163 was carried out as described by Carraway et al. [5]. Both DNA strands were sequenced using either an M13 specific 17-mer primer (New England Biolabs) or primer MM-6 (5-CCCTGCCCACTTCAA-3) which allows the sequence upstream of the *dam* gene to be determined.

Results and Discussion

Plasmid pMQ3 contains a 6.1 Kb insert of *Escherichia coli* chromosomal DNA containing both the *dam* and *trpS* genes [2]. Methylase assays of strains carrying either pMQ3 or its parent plasmid, pLC13-42, all show a relative methylase activity approximately tenfold over a *dam*$^+$ control strain, as shown in Fig. 1. In contrast, the methylase activity of a strain carrying p*dam*118, which is also derived from pLC13-42, showed only a one- to threefold increase (Fig. 1). To rule out a specific plasmid effect, the *Eco*RV-*Pvu*II fragment of p*dam*118 was recloned into pBR322, as described in Materials and Methods. Methylase assays of strains bearing the recombinant plasmid, pMQ148, again showed only a one- to threefold increase.

In order to determine whether a difference in copy number could be responsible for the low methylase activity of pMQ148, we measured the β-lactamase activity of pMQ148 and pMQ3 (Table 1). The relative copy number of pMQ148 was about twice that of pMQ3.

Plasmid pMQ133, containing the 1.85 Kb *Pst*I-*Sal*I fragment of pMQ3, shows a relative methylase activity of approximately tenfold; this suggests that the 1.85 Kb fragment contains *cis*-acting sequences for *dam* gene activity. Since pMQ133 contains additional chromosomal sequences both upstream and downstream compared with the pMQ148 insert, which might be responsible for the enhanced methylase activity, we subcloned the 1.24 *Pst*I-*Pvu*II fragment of pMQ133. The chromosomal insert of the resulting plasmid, pMQ162, has the same 3-prime end as that of pMQ148, but contains extra bases upstream compared with the 5-prime end of pMQ148 (Fig. 1). The relative methylase activity of pMQ162 was also about tenfold (Fig. 1), which again suggests that the required *cis*-acting sequences are present in the 1.24 Kb fragment.

The sequence of the *Pst*1-*Bam*H1 region containing the 346 bases 5-prime to the ATG of the *dam*

Table 1. Determination of relative copy number by measurement of β-lactamase activity[a]

Plasmid	β-Lactamase units	
	Avg.	Range
pMQ3	96	75–117
pMQ148	186	144–228
pBR322	108	55–160

[a] β-Lactamase assays were performed as described in Materials and Methods. Enzyme activity was measured in the *dam*-3 strain GM33 for all plasmids. Results are the averages of two to four assays.

gene is shown in Fig. 2. This sequence is present in pMQ162, pMQ133, and pMQ3. In p*dam*118, only the sequence 3-prime from base 229 is the same as in the other plasmids mentioned above. Upstream of base 229 in p*dam*118 is a sequence identical with bases 3041-3119 of pBR322. This sequence is bounded by —GATC— sites. Since the p*dam*118 backbone is composed of bases 2066-(4361)-375 of pBR322, the plasmid contains a 78 bp duplication. Presumably, the duplication was the result of a cloning artifact during the construction of p*dam*118 which employed *Sau*3A and *Mbo*I partial digests [4]. The increased production of methylase by plasmids such as pMQ162 is, therefore, due to the additional upstream 229 bases of *E. coli* chromosomal DNA.

After completion of this work, Jonczyk et al. [11] reported the same result with p*dam*118, as described here. Unlike our results, however, they did not detect increased expression due to the upstream 346 bases but found that the main promoter activity for *dam* came from a promoter located about 1.5 Kb upsteam in the *aroB* gene. The basis for the discrepancy between the two results is not yet known.

In order to assay for *dam* expression more easily and to rule out methylase inactivation as a cause of the low activity of pMQ148 and p*dam*118, we constructed derivatives of plasmids pMQ133, p*dam*118, and pMQ148 in which the *lacZ* gene was fused to the *dam* regulatory region as described in Materials and Methods. The results of β-galactosidase assays on strains containing these *dam-lacZ* fusion plasmids are presented in Table 2. It is clear that the pMQ133 derivative, pMQ139, has higher β-galactosidase activity than either the p*dam*118 derivative pMQ132, or the pMQ148 derivative pMQ159.

It was of interest to determine whether *dam* expression was affected by mutations in DNA repair genes, since *dam recA, dam recB,* or *dam recC* double mutants are lethal [14, 15]. We first assayed β-galactosidase activity of pMQ139 in both dam^+ and dam^- strains in order to determine whether the absence of a functional methylase affected *dam* expression. As shown in the first two lines of Table 3, no difference in enzyme activity was found. β-Ga-

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1         11        21        31
CTGCAGCTGAGCAGTTCCTCTAACTACGACAACCTGAACG
 PstI

41        51        61        71
GTTGGGCGAAGAAAGAGAATCTGAAAAACTACGTTGTCTA

81        91        101       111
TGAAACGACGCGTAATGGTCAGCCGTGGTATGTCCTGGTT

121       131       141       151
TCTGGCGTGTACGCTTCGAAAGAAGAGGCGAAAAAAGCGG

161       171       181       191
TATCTACATTGCCAGCAGATGTCCAGGCCAAAAACCCGTG

201       211       221       231
GGCGAAACCGCTGCGTCAGGTACAGGCCGATCTGAAGTAA
                              pdam118

241       251       261       271
TCAAGGTTATCTCCCGCAATGGTTTATCGTTGCGGGAGTT

281       291       301       311
GCCTGAAGCGCTGGATGCTGTCGGAGCTTTCTCCACAGCC

321       331       341  ***
GGAGAAGGTGTAATTAGTTAGTCAGCATGA
```

Fig. 2. DNA sequence of *Escherichia coli* DNA upstream of the *dam* gene. The sequence from the *Pst*I site (Fig. 1) to the ATG of the *dam* gene (asterisks) is shown. Plasmid *pdam*118 contains the sequence from base 229 onward.

Table 2. β-Galactosidase assays of strains containing *dam-lacZ* gene fusion plasmids[a]

dam-lacZ fusion plasmid	dam^+ parent plasmid	β-Galactosidase (units)
pMQ139	pMQ133	39
pMQ132	pdam118	3.0
pMQ159	pMQ148	2.5

[a] β-galactosidase assays were performed in whole cells as described in Materials and Methods. Assays were performed in strain GM1855[del(*lac-pro*)]. Each *dam-lacZ* gene fusion plasmid was derived from the corresponding dam^+ plamid as described in Materials and Methods.

Table 3. β-Galactosidase activity in repair-deficient strains carrying plasmid pMQ139[a]

Strain	Relevant genotype	β-Galactosidase
GM210	Wild type	85
GM1855	*dam-4*	76
GM2355	*recA56*	51
GM1343	*recB21 recC22*	56
GM1390	*recA200* (Ts)	156 (30°C)
		77 (42°C)
GM1984	*recB270* (Ts)	62 (42°C)
		47 (42°C)
GM1389	*recC271* (Ts)	39 (30°C)
		50 (42°C)
GM1379	*recA*441 *lexA*51 (Def)*sulA*211	121 (30°C)
		120 (42°C)
GM1377	*recB21 recC22 sbcA23*	128
GM1388	*recB21 recC22 sbcB15*	161
GM1380	*recA*430	103
GM1980	*lexA1* (Ind⁻)	104
GM1382	*uvrD502*	135
GM1378	*mutL451*	110
GM1381	*mutS453*	106

[a] All strains are del(*lac-pro*) and contain plasmid pMQ139, which carries a *dam-lacZ* fusion. β-Galactosidase activity was measured from whole cells as described in Materials and Methods. Cells were grown at 37°C unless stated otherwise.

lactosidase activity of pMQ139 was then assayed in a variety of DNA repair-deficient strains, and the results are presented in Table 3. No significant effect on the level of β-galactosidase activity was seen in strains carrying mutations in *recA, recB, recC, recBC, recBC sbcA, recBC sbcB, recA441 lexA51 sulA211, recA430, lexA, uvrD, mutL,* and *mutS*. This suggests that any interaction between the *dam* gene and other DNA repair genes does not affect expression of the *dam* gene on a multicopy plasmid.

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