

Phenotypic Reversal in *dam* Mutants of *Escherichia coli* K-12 by a Recombinant Plasmid Containing the *dam*⁺ Gene

JUDY A. ARRAJ AND M. G. MARINUS*

Department of Pharmacology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

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A recombinant plasmid, pMQ3, carrying the *dam* gene of *Escherichia coli* K-12, was constructed and transformed into *dam*⁺ and *dam*⁻ strains. Both *dam*⁻ and *dam*⁺ strains containing pMQ3 showed a wild phenotype for all traits, including mutation rate, except for a 10-fold increase in DNA adenine methylase activity.

The *dam* gene of *Escherichia coli* K-12 is believed to be the structural gene for a DNA adenine methylase (12, 15, 17), which modifies the -GATC- sequences (13) in newly synthesized daughter strands of DNA at or near the replication fork (3, 14). In addition to the primary phenotype (undermethylation and lack of DNA adenine methylase activity [17]), *dam* mutants display a pleiotropic phenotype (16; Marinus, in R. Borchardt, C. Creveling, and E. Usdin, ed., *Transmethylation*, in press) which suggests that DNA adenine methylation or the methylase may have a role in several cellular functions (16; Marinus, in press). One proposed function of the *dam* DNA methylase and modified or unmodified -GATC- sequences is that methylation allows a mismatch repair system to discriminate between newly synthesized unmethylated daughter strands and methylated parental strands at the replication fork (7).

We have cloned the *dam* gene and present data to show that a plasmid containing a functional *dam* gene in a *dam*⁻ strain reverses the phenotypes associated with *dam* mutants. This first step should now allow new and defined mutations to be constructed and should aid in the study of *dam* gene regulation.

From a plasmid constructed by Herman and Modrich that carried a *Pst*I fragment containing the *dam* gene (personal communication), we subcloned (2, 20) the smallest *Eco*RI fragment containing the *dam* gene into pBR325 (from F. Bolivar). The resultant plasmid, pMQ3, was isolated by screening tetracycline-resistant transformants of GM237 (Table 1) for chloramphenicol sensitivity and 2-aminopurine (2-AP) resistance since *dam* mutants are sensitive to 2-AP (8). pMQ3 contains a 6.1-kilobase *Eco*RI insert in the *Eco*RI site of pBR325 (Fig. 1). In contrast to pBR325, pMQ3 is resistant to cleavage by *Mbo*I when propagated in a *dam* mutant. Since cleavage by *Mbo*I is prevented by the action of the *dam* gene product, pMQ3 must

contain a functional *dam* gene. We have localized the *dam* gene on the smaller *Sal*I-*Eco*RI fragment as indicated in Fig. 1 by subcloning this fragment into pBR322 and determining the presence of a functional *dam* gene. All attempts to clone fragments containing one or two *Bam*HI ends and a functional *dam* gene were unsuccessful, and it was concluded that the *Bam*HI site is within the *dam* gene.

The larger *Sal*I-*Eco*RI fragment, containing a *Kpn*I site, carries the *trpS* gene. Strain 42c (from L. A. Isaksson) carries a mutation in *trpS* that results in a temperature-sensitive phenotype for growth. Introduction of pMQ3 into strain 42 allows growth at high temperature, indicating the presence of a functional *trpS* gene. In addition, the *trpS* gene has recently been sequenced (9) and has been found to contain a single *Kpn*I site (C. V. Hall, personal communication). We have detected a new protein in *in vivo*-labeled cells of pMQ3 but not of pBR325 (data not shown). It has an apparent molecular weight of 37,000 and is the *trpS* gene product. We have not yet succeeded in detecting the *dam* gene product.

Confirmation of the presence of a functional *dam* gene was obtained by *in vitro* and *in vivo* methylation assays (1, 15, 17), and the results are summarized in Table 2. Methyl transfer occurred with DNA from the *dam*⁻ strain, but neither the *dam*⁺ strain nor the pMQ3/*dam*⁻ strain. In addition, the specific activity of the methylase in the pMQ3/*dam*⁻ strain was about 10-fold higher than in the *dam*⁺ strain (Tables 2 and 3). There was less than 10 pmol of methyl group transfer into DNA with *dam*-3 crude extracts.

The ability of pMQ3 to revert the pleiotropic phenotypes of *dam* mutants was tested, and the results for several of these phenotypes are summarized in Table 2. Except for the increased level of methylase activity, pMQ3 containing *dam*⁻ strains showed a wild phenotype for all of

TABLE 1. *E. coli* K-12 strains^a

Strain	Sex	Genotype
GM119	F ⁻	<i>dam-3 dcm-6 metB1 galK2 galT22 lacY1 tsx-78 supE44 (thi-1 tonA31 mtl-1)?</i>
GM215	F ⁻	<i>dam-3 end-1 rns-1 supE44 (thi-1 rel-1)?</i>
GM237	F ⁻	<i>dam-3 recA1 sin-1 mut-454 thr-1 leuB6 proA2 his-4 metB1 lacY1 galK2 ara-14 tsx-33 thi-1 deoB16 supE44 rpsL260</i>
GM1635	F ⁺	F- <i>pro</i> ⁺ <i>lacZ::Mu cts62Xcam/dam-4 Δ(lac-pro)X111 thi-1 supE44</i>
GM1737	F ⁻	<i>dam-4 cysG::Tn5 mal-354 tsx-354</i>

^a Strain GM119 was used for large-scale plasmid preparations (5), and GM215 was used for preparation of plasmid DNA from 10-ml cultures (6). Strain GM237 was also used for plasmid preparation when it was necessary to use a *recA*⁻ strain. The Tn5 insertion in GM1737 was used to transfer *dam* alleles between strains by P1 transduction. The bacteriophage Mu plasmid was obtained from A. Bukhari, and the *cysG::Tn5* insertion from C. Berg.

those tested. pMQ3 also reverses the sensitivity of *dam* mutants to alkylating agents and UV light and reverses the inviability of *dam*⁻*recA*⁻(Ts) or *dam*⁻*recB*⁻(Ts) or C⁻(Ts) strains at the nonpermissive temperature (data not shown). Table 2 also presents two new aspects of *dam* mutants. One is that there is an increased frequency of spontaneous (*tonB-trp*) deletion formation, and the other is that precise excision of Tn5, but not phage Mu, is increased. Both of these traits are reversed by pMQ3.

There is an increased spontaneous mutability in *dam* mutants (18). The presence of pMQ3 in *dam*⁻ bacteria reduces the frequency of missense (Rif^r, Nal^r; Table 2), frameshift (data not shown), and deletion (*tonB-trp*; Table 2) muta-

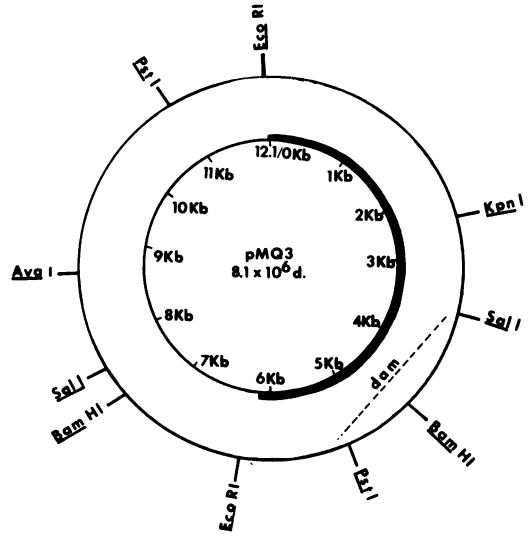


FIG. 1. Restriction cleavage map of pMQ3 with the location of the insert carrying the *dam* gene. The boldface line represents the cloned insert into pBR325 carrying the *dam* gene. The unique sites for *Pst*I, *Ava*I, *Sal*I, *Bam*HI, and *Eco*RI on the pBR325 part of the plasmid are drawn as described by Prentki et al. (19). The unique sites for *Kpn*I, *Sal*I, *Bam*HI, and *Pst*I on the insert were determined from agarose gels. Restriction endonucleases were obtained from New England Biolabs, Bethesda Research Laboratories, or Boehringer-Mannheim Corp. Reaction conditions were those specified by the supplier. When samples were digested with two or more enzymes, the one requiring the lowest salt concentration was done first, or the sample was diluted to the required salt concentration. Electrophoresis in 0.6 to 1.5% agarose (Seakem) gels was in Tris-acetate buffer, pH 7.8 (10), containing 0.5 μg of ethidium bromide per ml (21). Kb, Kilobases. d, Daltons.

bility to normal levels. pMQ3 had little effect on the spontaneous mutability in *dam*⁺ strains (Table 3) or in *recA13* derivatives of AB1157 (data not shown).

TABLE 2. Effect of pMQ3 on properties of *dam* mutants

Strain	mol% of 6-methyladenine	Methylase activity		2-AP ^b	λ ind ^{-c}	F-lac ^d	Mutation frequency			Precise excision ^f	
		Sp act ^a	In vitro ^a				Rif ^r	Nal ^r	Δ(<i>tonB-trp</i>) ^e	Tn5	Mu
<i>dam</i> ⁺	1.5	1	391	R	1	1	1	1	1	1	1
<i>dam-3</i>	≤0.06		18,693	S	38	15	124	74	11	10-100	1
pMQ3/ <i>dam-3</i>	1.5	11.8	394	R	0.25	0.3	5	1	1	3	1

^a Specific activity is normalized to 1 for the wild type. In vitro activity is counts per minute per milligram of protein, where transfer of radioactive methyl groups is measured (1, 21)

^b Resistance (R) or sensitivity (S) to 100 μg of 2-AP per ml.

^c Spontaneous induction in logarithmic-phase cultures (1).

^d Frequency of Lac⁻ cells, which are permissive for phage fd, as a percentage of total cells in overnight broth cultures (18).

^e Isolated as resistant to φ80vir, ColV, and B and tryptophane requiring.

^f For Mu, *lacZ* reversion was scored. For Tn5, reversion of *cysG::Tn5* to prototrophy was scored.

TABLE 3. Mutability of *E. coli* strains containing the cloned *dam* gene

Strain	Relevant genotype	Relative <i>dam</i> methylase activity	Mutation frequency ^a (10 ⁶)	
			Rif ^r	Val ^r
AB1157	<i>dam</i> ⁺	1	1.7	6.9
pBR322/AB1157	— <i>dam</i> ⁺	0.87	3.3	8.7
pMQ3/AB1157	<i>dam</i> ⁺ / <i>dam</i> ⁺	9.8	2.1	16.5
pGG503/AB1157	<i>dam</i> ⁺ / <i>dam</i> ⁺	42	20	40
GM113	<i>dam</i> -3		113	628

^a Mutation frequency was measured as the mutants/viable count ratio. Results are the average of one to four experiments, each with five independent cultures. Cells were grown overnight in brain heart (BH) broth. Plasmid-containing cultures contained 15 µg of tetracycline per ml. The cells were plated on BH agar and BH agar containing 100 µg of rifampin per ml. To test for resistance to valine, the cells were suspended in minimal media and plated on minimal plates. Minimal plates contained 40 µg of valine per ml.

The result obtained by Herman and Modrich (11) showing that pGG503, which also contains the *dam* gene, conferred hypermutability contrasts sharply with the results for pMQ3, which does not alter mutability. The discrepancy between these results may be because of the level of DNA adenine methylase produced by each plasmid. Table 3 shows that pGG503-containing strains produce about four times more enzyme activity than pMQ3-containing strains. Measurements of *dam* gene activity and mutability in cells in which *dam* gene activity can be varied over a wide range may answer this question.

Herman and Modrich have reported that pGG503 produces a 10 to 20-fold elevation of DNA adenine methylase activity in *recA*⁺ hosts, but a 50-fold elevation in a *recA*⁻ host (12). In contrast, we found that the 10-fold elevation of enzyme activity of pMQ3 and the 40-fold elevation of activity of pGG503 are the same in *recA*⁺ and *recA*⁻ hosts (data not shown).

During the completion of this work, Brooks et al. (personal communication) isolated and sequenced the *dam* gene. Their data indicate that *dam* is the structural gene for the methylase and that a *Bam*HI recognition site is present within the gene.

The ability of pMQ3 to reverse the *dam*⁻ phenotypes without causing any apparent alterations in the wild phenotype other than an increased synthesis of DNA adenine methylase together with the data obtained by Brooks et al. should allow new and defined mutations to be constructed and the study of the regulation of the *dam* gene to begin.

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