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The *dam* and *dcm* strains of *Escherichia coli* – a review

(DNA methylation; recombinant DNA; restriction; gene regulation)

B.R. Palmer^a and M.G. Marinus^b

^aDepartment of Plant and Microbial Sciences, University of Canterbury, Christchurch, New Zealand; and ^bDepartment of Pharmacology, University of Massachusetts, Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA. Tel. (1-508) 856-3330

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SUMMARY

The construction of a variety of strains deficient in the methylation of adenine and cytosine residues in DNA by the methyltransferases (MTases) Dam and Dcm has allowed the study of the role of these enzymes in the biology of *Escherichia coli*. Dam methylation has been shown to play a role in coordinating DNA replication initiation, DNA mismatch repair and the regulation of expression of some genes. The regulation of expression of *dam* has been found to be complex and influenced by five promoters. A role for Dcm methylation in the cell remains elusive and *dcm*⁻ cells have no obvious phenotype. *dam*⁻ and *dcm*⁻ strains have a range of uses in molecular biology and bacterial genetics, including preparation of DNA for restriction by some restriction endonucleases, for transformation into other bacterial species, nucleotide sequencing and site-directed mutagenesis. A variety of assays are available for rapid detection of both the Dam and Dcm phenotypes. A number of restriction systems in *E. coli* have been described which recognise foreign DNA methylation, but ignore Dam and Dcm methylation. Here, we describe the most commonly used mutant alleles of *dam* and *dcm* and the characteristics of a variety of the strains that carry these genes. A description of several plasmids that carry *dam* gene constructs is also included.

INTRODUCTION

Escherichia coli K-12 strains containing mutations in genes encoding the methylation of adenine and cytosine in DNA were first isolated in 1972 (Marinus and Morris, 1973). These genes were designated *dam* and *dcm*, respectively. The presence of *dam* and *dcm* mutations in a strain was identified by an assay which detected the methylation of mutant strain DNA by a wt cell extract, indicating the

lack of normal methylation. This observation was confirmed by chemical degradation of isolated chromosomal DNA from mutated isolates, followed by thin layer chromatography of the DNA degradation products to determine the presence or absence of methylated bases. Subsequent mapping studies located the *dam* gene at 74 min and *dcm* at 43 min on the *E. coli* K-12 chromosome map (Marinus, 1973; Bachmann, 1990) and further study yielded data which suggests the roles that these two genes

Correspondence to: Dr. B.R. Palmer, Department of Plant and Microbial Sciences, University of Canterbury, Private Bag 4800, Christchurch, New Zealand. Tel. (64-3) 366-7001, Fax (64-3) 364-2083; e-mail: palmer@botn.canterbury.ac.nz

Abbreviations: 2-AP, 2-aminopurine; 6-meA, 6-methyladenine; aa, amino acid(s); bp, base pair(s); Cm, chloramphenicol; CRP, cAMP-receptor protein; Dam, DNA adenine MTase; *dam*, gene encoding Dam; Dcm, DNA cytosine MTase; *dcm*, gene encoding Dcm; EtdBr, etidium

bromide; IN, regional chromosomal inversion; IPTG, isopropyl-β-D-thiogalactopyranoside; kb, kilobase(s) or 1000 bp; Km, kanamycin, Mb, 10⁶ bp; MTase, DNA methyltransferase(s); N, A or C or G or T; nt, nucleotide(s); Oc, ochre suppressor mutation; P, promoter; ^R, resistant; R, A or G; R-M, restriction-modification (systems); ss, single strand(ed); Tc, tetracycline; Tn, transposon; *tsp*, transcription start point(s); *urf*, unidentified reading frame; Vsp, very short patch, W, A or T; wt, wild type; XGal, 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside; [], denotes plasmid-carrier state; ::, novel junction (fusion or insertion); Δ, deletion.

have to play in the biology of *E. coli* (Marinus and Morris, 1974).

METHYLATION BY Dam AND Dcm

(a) Biological function for Dam methylation

Studies of the role of the Dam methylation in the biology of the *E. coli* cell have revealed that the *dam* gene product plays a role in the repair of DNA mismatches. Dam methylation also appears to be involved in the regulation and coordination of several other cell processes, including DNA replication and the modulation of gene expression.

(1) Mismatch repair

Dam methylation is an integral part of the methyl-directed mismatch repair system (Meselson, 1988; Lahue et al., 1989; Modrich, 1989). Dam methylates the N⁶ position of adenine in the sequence GATC. This methylation occurs shortly after the new DNA strand has been synthesized at the replication fork. As a result a stretch of DNA close to the replication fork remains hemimethylated for a short time after replication. This allows the mismatch repair system to discriminate between the template strand and the nascent strand and correct any misincorporated bases in the newly synthesized strand. Dam methylation proceeds following this repair (Campbell and Kleckner, 1990). Bergerat et al. (1989) have presented evidence that Dam methylates DNA by a processive sliding mechanism rather than randomly methylating available hemimethylated sites. In strains with a mutation in the *dam* gene all GATC sites are unmethylated (Russel and Hirata, 1989), therefore the mismatch repair system cannot differentiate between the template and daughter strand. Mismatch repair still occurs in these strains but repair occurs on either strand. As a result *dam* strains have an increased spontaneous mutation frequency (Marinus and Morris, 1975). Strains overexpressing Dam also have elevated mutation rates (Marinus et al., 1984) due to rapid methylation of newly-replicated GATC sites reducing the effectiveness of mismatch correction by the mismatch repair system in surrounding nascent DNA.

(2) DNA replication

Initiation of a new round of *dnaA*-dependent chromosome replication from *oriC* in minichromosomes occurs only at low efficiency without full methylation of the eleven GATC sites which are located within the minimal *oriC* region (Messer et al., 1985; Smith et al., 1985). Flow cytometry measurements of DNA from *dam* mutant strains show they have uncoordinated DNA replication initiation compared to wt strains (Boye et al., 1988). Ogden et al. (1988) have described experiments suggest-

ing that a hemimethylated region of DNA is required for the binding of a fragment containing the *E. coli oriC* region to membrane sites in dividing cells. This suggests the methylation of the *oriC* region may be an important factor in coordinating DNA replication initiation, chromosome partition and cell division.

(3) Modulation of gene expression

Several *E. coli*, bacteriophage and transposon genes which contain GATC sites in their promoter sequences are regulated by Dam methylation (Sternberg, 1985; Barras and Marinus, 1989). The passage of the replication fork through the region of the chromosome containing each of these genes leads to an increase or reduction of the amount of transcription from these promoters (e.g., *dnaA* promoter, Tn10 P_{in} promoter). While the modulation in activity effected by methylation is usually modest, on the order of 2–6 times, this may have a significant effect on the action of a particular gene product or pathway (Sternberg, 1985). For example, Tn10 and Tn5 exhibit bursts of transposition, deletion or inversion following the passage of the replication fork through their genomes. This type of regulation obviously plays an important role in spread of these elements to new sites. Regulation of the *pap* operon, encoding pyelonephritis-associated pili, has been shown to involve the differential methylation/nonmethylation of two Dam sites in the operon regulatory region (Van der Woude et al., 1992). Evidence for the involvement of Dam in the regulation of *daa* (F1845 pili), *fae* (K88 pili), *fan* (K99 pili), *fim* (type I pili) and *sfa* (S pili) has also been recorded (Van der Woude et al., 1992).

(4) Under-methylation

The results of Ringquist and Smith (1992) have shown that approx. 0.2% of the estimated 18 000 GATC sites (i.e., 36) are fully unmethylated even in wt *dam*⁺ *E. coli* strains and are susceptible to *MboI* cleavage. Unmethylated Dcm sites were also detected. The number and location of *MboI*-sensitive Dam sites was shown to change dependent on growth medium and stage of growth, suggesting that DNA-binding proteins may be protecting specific regions of the chromosome (Ringquist and Smith, 1992). This has been confirmed by Wang and Church (1992) who showed that in vivo protected Dam sites occurred in the 5' non-coding regions of seven *E. coli* operons (*mtl*, *cdd*, *flh*, *gut*, *car*, *psp* and *fep*). Four operons (*mtl*, *cdd*, *flh* and *gut*) were shown to have sequences closely matching the consensus binding site of the cAMP-receptor protein (CRP). A strain containing a deletion in the *crp* gene (encoding CRP) was found to have fewer in vivo protected Dam sites (Wang and Church, 1992). Overproduction of Dam in a strain carry-

ing the plasmid pTP166 (Marinus et al., 1984) was found to increase but not complete GATC site methylation (Ringquist and Smith, 1992). However in our hands AB1157[pTP166] has fully Dam-methylated DNA (Fig. 1). This may be a host strain effect as Ringquist and Smith (1992) used an EMG2 background. EMG2 and AB1157 have several differences in gross chromosomal structure (Perkins et al., 1993) and EMG2 is $F^+\lambda^+$, while AB1157 is $F^-\lambda^-$. These differences may affect the response of the strains to Dam overexpression or their relative degrees of Dam overexpression. Alternatively our experiment used *NdeII*, an isoschizomer of *MboI*, and

any difference in the flanking sequence preferences of these enzymes may affect the activity of these enzymes on partially methylated DNA contributing to the observed differences.

(5) Expression of *dam*

The expression of the *dam* gene has been studied intensively in the past few years. Evidence for weak promoter activity (denoted *P5*) just upstream from the *dam* *tsp* has been available for some time (Arraj et al., 1990; Wu et al., 1992). However with the construction of *dam::lacZ* fusions on low-copy-number plasmids, Løbner-Oleson et al. (1992) have shown that *dam* expression is influenced by at least five promoters (Fig. 2). The strongest of these are two closely linked regions (*P1* and *P2*) which lie 5' to the genes *aroK* and *aroB*. Two further promoters which lead to some transcription of *dam* mRNA, *P3* and *P4*, lie at the 3' end of the functionally cryptic *urf74.3* (Løbner-Oleson et al., 1992). The *urf74.3* gene lies between *dam* and *aroKB* and all four genes are transcribed in the same direction (Jonczyk et al., 1989; Løbner-Oleson et al., 1992). Quantification of Dam protein in *E. coli* laboratory strains (e.g., AB1157, C600, W3110) using rabbit anti-Dam antibodies as a probe to Western blots of total cellular protein produced estimates of approx. 130 molecules per cell in exponential phase cultures (Boye et al., 1992). It seems likely that the construction of chromosomal *dam::lacZ* fusions will allow rapid accumulation of data on changes in the level of *dam* expression in response to different phases of growth and alterations in growth medium contents.

(6) Evolutionary aspects

From an evolutionary point of view, methylated GATC sequences have been detected in certain Gram⁺ and Gram⁻ bacteria, as well as in archaeobacteria (Brooks et al., 1983; Barbeyron et al., 1984; Hattman et al., 1985; Lodwick et al., 1986; Bolstad and Jensen, 1993), but presumably some of these are associated with restriction systems. There is aa sequence homology among the various

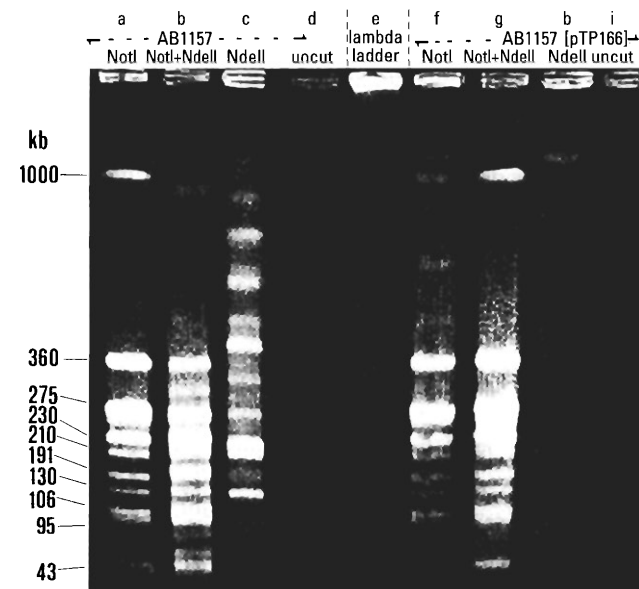


Fig. 1. The effect of Dam over-expression on unmethylated GATC sites. DNA was purified from *E. coli* strains AB1157 (lanes a–d) and AB1157[pTP166] (lanes f–i) using the method of Ringquist and Smith (1992) (for a description of pTP166, see section h). Both strains were grown in LB broth to an A_{600} of 1.0, AB1157[pTP166] was grown in the presence of 50 μ g ampicillin/ml and 1 mM IPTG. The DNA was digested as indicated and subjected to pulsed-field gel electrophoresis in a 1% agarose gel on a Bio-Rad CHEF Mapper™ using the parameters: run time 26 h 40 min, 6 V/cm, initial switch time 6.75 s, final switch time 1 min 33.69 s, included angle 120° and 0.5 \times TBE buffer. The gel was stained with EtdBr for 30 min and destained in water for 1 h. The restriction endonuclease *NdeII* is an isoschizomer of *MboI*, neither enzymes digest Dam methylated (nor hemimethylated) GATC sites. The *NotI* digest in lane a allows DNA fragments to be related to the *NotI* physical map of the *E. coli* chromosome (Smith et al., 1987; Perkins et al., 1993). Bands present in lane b, but not in lane a, indicate unmethylated GATC sites at discrete locations. Bands will only be seen if the majority of DNA molecules are unmethylated at a particular GATC, sporadic unmethylated sites will be invisible under these conditions. AB1157[pTP166] appears to have fully Dam-methylated GATC sites; comparing lanes g and f, no extra bands appear in lane g and this is confirmed in a less sensitive way by comparing lanes c and h. Little or no EtdBr-derived fluorescence is visible in lanes d and h, a reproducible phenomenon, possibly due to resistance of undamaged nucleoids to EtdBr staining. Lane e contains λ DNA concatemers (Bio-Rad) as molecular size standards. TBE is 89 mM Tris-borate/89 mM boric acid/1 mM EDTA pH 8.0.

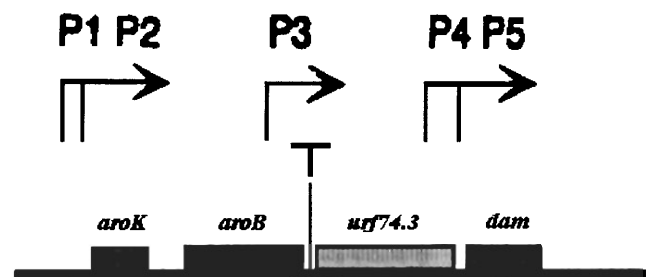


Fig. 2. A genetic map for the region of the *E. coli* K-12 chromosome containing the *dam* gene (adapted from Løbner-Olesen et al., 1992). The five promoters known to affect *dam* expression are shown. *P1*–*P5* indicate characterised promoters; T denotes a transcriptional terminator site.

Dam MTases so far identified suggesting that there is a common evolutionary origin for all Dam MTases. The *dam* gene appears to be one of the few genes that is distributed relatively universally among the Enterobacteriaceae (Barbeyron et al., 1984). Based on nt sequencing, a number of class-II MTases share significant sequence similarity with Dam (Lauster et al., 1987).

(7) Other features

It is interesting to note that several *E. coli* phages have acquired a copy of the *dam* gene, presumably to augment the host gene during infection, while Dam methylation has been shown to play an important role in the packaging of P1 phage DNA (Sternberg and Coulby, 1990). Also the recently described retron from *E. coli*, retron Ec67, contains a gene with functional homology and sequence similarity to *dam* (Hsu et al., 1990). However, there are many bacterial species that do not contain methylated GATC sequences. What mechanism do such bacteria (and all eukaryotes) use for strand discrimination if temporary Dam hemimethylation does not occur? One possibility is the persistence of single-strand breaks in the lagging DNA strand due to discontinuous replication (Claverys and Lacks, 1986).

(b) Biological function for Dcm methylation

Originally the only phenotype observed in *dcm* mutants was susceptibility to restriction by the restriction systems of the IncN plasmids (e.g., *EcoRII* endonuclease of plasmid N3; Hattman, 1977). There is evidence that the Dcm and the *EcoRII* MTase may be derived from a common ancestor, since they share a large degree of sequence similarity (Lauster et al., 1989) and are functionally very similar.

(1) Very short patch repair

Deamination of 5-methyl cytosine (5-mC) results in the formation of a thymine base. It has been observed that the recognition sites of Dcm (CCWGG) are hotspots for mutation, specifically C→T transitions at the 3' C due to deamination of 5-mC. A repair system which recognises and repairs T:G mismatches at these sites by the formation of short (< 10 bp) excision tracts has been described (Lieb, 1983; Lieb et al., 1987). This type of repair has been termed very short patch (Vsp) repair and was thought to require the gene products of *mutL* and *mutS*, and the Dcm MTase (Lieb, 1987; Lieb and Bhagwat, 1988). Recent studies of the cloned *dcm* gene and surrounding regions indicate that the product of a gene, *vsr*, partially overlapping the *dcm* gene, is required in Vsp repair rather than Dcm (Bhagwat et al., 1988; Sohail et al., 1990). The *vsr* gene product has since been shown to be a DNA mismatch endonuclease which recognises the T/G mismatches that result from 5-mC deamination

at Dcm sites (Hennecke et al., 1991). It may be that the *dcm-6* allele which abolishes the Vsp repair pathway also affects *vsr* or is a polar mutation. Expression of *vsr* would appear to be coordinated with that of *dcm*, which would seem to be desirable (Sohail et al., 1990). This may resolve the problem of Dcm's involvement in repair of errors brought about by its presence in the cell, which seems to be an unnecessary redundancy.

(2) Possible roles

It remains uncertain what the function of the *dcm* gene is. It is possible that its presence on the *E. coli* chromosome is the result of a recent recombination event between the chromosome and an IncN plasmid and is of no real biological significance. However, the observation that genes that contain a Dcm recognition site within their promoters may be regulated by Dcm methylation, may have some bearing on *dcm*'s significance. One such gene is *lexA*, which is of prime importance to the cell and its ability to respond to DNA damage. A Dcm methylation site overlaps the *lexA* promoter (Miki et al., 1981) but an effect on *lexA* expression by varying Dcm levels is yet to be shown. Some Dcm sites remain unmethylated in Dcm⁺ cells (Ringquist and Smith, 1992), suggesting the protection of these sites by DNA binding molecules or DNA topology.

(c) Uses for methylation-deficient strains

With the widespread use of recombinant DNA technology one of the primary uses of methylation-deficient strains is for the propagation of DNA molecules so that they lack methylation at either or both Dam and Dcm sites. Such methylation can inhibit the digestion of DNA by restriction endonucleases which have recognition sites that contain or overlap Dam or Dcm recognition sites (e.g., *BclI*, *XbaI*, *EcoRII*). A detailed list of those endonucleases affected by DNA methylation has been compiled by Kessler and Manta (1990) and Nelson and McClelland (1992). By growing the particular molecule of interest in a suitable methylation-deficient strain this problem is readily overcome. Methylation, including the Dam system, was used to create very rare cleavage sites employing the Achilles' heel cleavage (AC) method (Grimes et al., 1990; Koob and Szybalski, 1994).

Some reports have suggested that considerable residual methylation occurs in the commonly used methylation-deficient strains. A study which used an extremely sensitive method to detect methylation showed that for *dam* strains, even with the original mutant alleles, methylation occurs at less than one methyl group per chromosome copy and even less in mutants containing insertions in the *dam* gene (Russell and Hirata, 1989).

Transformation of *Streptomyces*, *Bacillus* and *Paracoccus* species is greatly improved by the use of DNA

lacking Dam and Dcm methylation. For example, MacNeil (1988) found that preparation of shuttle-vector plasmid DNA in a *dam*⁻ *dcm*⁻ *E. coli* strain increased the frequency of the *Streptomyces* transformation by 400–10 000-fold relative to modified DNA.

DNA isolated from strains deficient in Dcm gives better results in Maxam and Gilbert DNA sequencing procedures because 5-meC is resistant to chemical attack by hydrazine. As a result 5-meC positions are missing from Maxam and Gilbert sequence patterns. DNA isolated from *dam* strains is not suitable for the chain termination method of DNA sequencing due to the number of ss breaks present in the DNA, presumably as the result of nicking by MutH protein (M. Carraway and M.G.M., unpublished results).

Mismatch repair of unannealed bases in primers used for site-directed mutagenesis can lead to preferential loss of the desired mutant species. Preparation of template DNA in *dam* strains alleviates this problem, as the template strand is unmethylated and is no longer resistant to mismatch repair. Site-directed mutagenesis can be performed in the normal way and the product transformed into either a *dam*⁻ or *dam*⁺ strain.

Strains deficient in Dam MTase are particularly susceptible to low level mutagens, resulting in the full induction of the damage-inducible or SOS repair system. With the availability of *lacZ* fusions to several of the damage-inducible (*din*) genes (Walker, 1984) it is a relatively simple matter to screen compounds for potential low level mutagenicity using a *dam* *din::lacZ* strain on a suitable medium and achieve a quite sensitive assay (Craig et al., 1984; Quillardet and Hofnung, 1987).

Methylation-deficient strains of *E. coli* K-12 were originally isolated in an effort to determine the role of DNA methylation in the biology of the organism. This continues to be one of the primary uses of these strains in trying to gain a better understanding of the roles that each system plays in the cell's overall organisation and regulation.

(d) Working with *dam*⁻ and *dcm*⁻ strains

(1) Storage of strains

It has been found that storing *dam* strains on agar medium (i.e., on plates or in slope or stab culture) for periods longer than approximately one month results in strain instability. This is presumably because of the increased (over wt) spontaneous mutation rates resulting from this mutations. The *dam* deficiency impairs the ability of the mismatch repair system to correct newly synthesized strand errors, increasing the spontaneous mutation rate to 8–250-fold greater than that of wild-type strains (Marinus and Morris, 1975). *dcm* strains have no detectable phenotype other than inability to carry out Vsp repair

and can be stored by normal methods, in our laboratories this is usually by the method detailed below.

The most suitable method of storage for these strains is to keep aliquots of cell suspensions in broth containing 40% glycerol at -70°C , under which conditions they are stable and viable for many years.

(2) Assays for the *Dam* phenotype

In order to determine the methylation state of the DNA of a particular *E. coli* strain it is important to be able to assay for the presence of methyl groups at particular positions on the bases of interest. This can be achieved by a number of methods. Marinus and Morris (1973) assayed for the addition of radioactively labelled methyl groups by wt strain extracts to unmethylated DNA detected by chemical degradation of the DNA followed by thin-layer chromatography. More sensitive and convenient methods for the detection of 6-methyladenine (6-meA) at GATC sites have since been developed. These include the differential digestion of methylated GATC sites by *DpnI*, *MboI* and *Sau3AI*. *DpnI* will only digest GATC sites containing 6-meA, *MboI* will only digest unmethylated GATC sites and *Sau3AI* cleaves regardless of the methylation state of GATC adenine residues. Sensitive methods for the detection of very rare DNA methylation have been developed by Russel and Hirata (1989) which can detect the very small amount of Dam methylation (<1 methyl group per chromosome copy) that occurs in *dam* mutant cells.

A simple method for testing a strain to identify it as a *dam* mutant is to check the ability of a drop of a cell suspension of 10^6 cells/ml to grow well on nutrient plates containing 100 μg 2-aminopurine (2-AP)/ml. A wt cell will give confluent growth while a *dam* strain will give at most several isolated colonies. The basis of the 2-AP sensitivity of *dam* strains is not fully understood, but it has been shown that 2-AP-resistant colonies arising from *dam* strains have mutations in either one of the genes for the Dam-directed mismatch repair proteins or in the as yet unidentified *sinA* locus (McGraw and Marinus, 1984). Gene fusions of the GATC-containing promoter P_{in} from *Tn10*, which is activated in *dam* strains, to a readily assayed gene such as *lacZ* also provide a simple test for a strain's *dam* status. The phage λ derivative λER60 (E.A. Raleigh, unpublished data) contains such a construct and *dam* *lacZ* λER60 lysogens produce blue colonies on plates containing XGal, while *dam*⁺ *lacZ* lysogens produce white colonies.

(3) Assays for the *Dcm* phenotype

As with *dam* strains, *dcm* strains can be assayed for Dcm MTase activity by testing for the addition of radioactively labelled methyl groups by wt strain extracts to

unmethylated DNA, detected by chemical degradation of the DNA followed by thin-layer chromatography (Marinus and Morris, 1973). However, a simpler method is to test the efficiency of plating of phage (e.g. λ_{vir}) propagated on the test strain (e.g., GM967) when plated on a strain (e.g., GM1212) containing a plasmid encoding the *EcoRII* restriction endonuclease which cleaves unmethylated Dcm sites, such as pN3 (May and Hattman, 1975). Phage grown on *dcm* strains have a low efficiency of plating when tested in this manner. Similarly DNA from *dcm* strains is cut by *EcoRII* while DNA from *dcm*⁻ strains is not.

(e) Restriction systems of *E. coli* and DNA methylation

E. coli cells have been shown to have restriction systems which serve to protect the cell from the potentially harmful effects of transfer of foreign DNA to the cell by the mechanisms of phage infection, plasmid transfer or transformation. These systems can be classified as one of two types depending on whether they recognise the presence or absence of a particular pattern of modification of the DNA, which is usually methylation of particular adenine or cytosine residues in short recognition sequences.

R-M systems are two component systems, consisting of a restriction endonuclease which catalyses cleavage of DNA at or near unmodified recognition sequences and a MTase which protects endogenous DNA by methylation of bases in the recognition sequence. The *hsd* genes of *E. coli* code for the classical K-12 R-M system of class I.

More recently another 'complementary' class of restriction system, the methyl-dependent restriction system, has been recognised (Blumenthal, 1989; Raleigh et al., 1989). These systems recognise DNA containing methylated adenine or cytosine at particular sequences and cleave this methylated foreign DNA. The loci *mcrA*, *mcrB*, *mcrC* and *mrr* have been shown to be responsible for systems of this type in *E. coli* K-12. The *mcrABC* loci restrict DNA with foreign cytosine methylation, while *mrr* restricts DNA with foreign adenine methylation (Heitman and Model, 1987) and also some types of cytosine methylation (Kelleher and Raleigh, 1991; Waite-Rees et al., 1991). Genes encoding *mcrBC* and *mrr*, along with those of the *hsdRMS* system, have been mapped at approx. 98.5 min on the *E. coli* K-12 map (Heitman and Model, 1987; Blumenthal, 1989; Dila et al., 1990). This region is now commonly called the 'immigration control region', as sequence data for this region suggests that the genes *hsdS*, *mcrB* and *C* have been recently acquired as they have a significantly lower G+C content than surrounding regions of the chromosome (Dila et al., 1990). *mcrA* maps at approx. 25 min within the genome of the cryptic prophage element $\epsilon 14$ (Blumenthal, 1989; Raleigh et al., 1989;

Hiom and Sedgwick, 1991). The specificities of each of these systems appears to be rather loose (Heitman and Model, 1987; Dila et al., 1990; Waite-Rees et al., 1991; Sutherland et al., 1992). A suggested consensus target site for the *McrBC* restriction enzyme is R^mC(N40-80)R^mC (Sutherland et al., 1992). The specificity of *McrA* appears to be C^mCGG (Raleigh and Wilson, 1986), while that of *Mrr* appears complicated despite extensive testing against a variety of MTases (Waite-Rees et al., 1991). DNA that is methylated by *E. coli* K-12 resident MTases Dam and Dcm is not affected by these restriction systems.

Strains with mutations in some or all of the restriction system genes described above have now been constructed. Derivatives that are also *dam*⁻ (e.g., GM4715) are available (B.R.P., E.A. Raleigh and M.G.M., unpublished data) and these strains will probably prove to be the most generally useful for manipulating recombinant DNA.

(f) Alleles of *dam* and *dcm*

A number of different mutant alleles of the *dam* gene are available, while the *dcm* allele in most commonly used *dcm* strains is *dcm-6*. The most commonly used *dam* alleles are *dam-3*, *dam-4*, *dam-13* and *dam-16*. Like *dcm-6*, *dam-3* and *dam-4* were induced by chemical mutagenesis (Marinus and Morris, 1973; Marinus and Konrad, 1976). The two *dam* alleles *dam-13* and *dam-16* were constructed by Tn9 insertion (Marinus et al., 1983) and replacement of part of the *dam* gene with a fragment encoding kanamycin resistance (Parker and Marinus, 1988), respectively. The characteristics of these alleles are outlined in Table I. All *dam* alleles form inviable strains in combination with mutations in the genes *recA*, *recB*, *recC*, *recJ*,

TABLE I
Various *dam* and *dcm* alleles

Allele ^a	Associated ^b antibiotic resistance	Methylation ^c level	Reference
<i>dcm-6</i>		nd	Marinus and Morris (1973)
<i>dam-3</i>	—	1/23 kb	Marinus and Morris (1973)
<i>dam-4</i>		1/14 kb	Marinus and Konrad (1976)
<i>dam-13::Tn9</i>	Cm ^R	1/10 Mb	Marinus et al. (1983)
<i>dam-16</i>	Km ^R	nd	Parker and Marinus (1988)

^aSee section f for a description of these alleles.

^bKm^R (25 µg/ml), Cm^R (10 µg/ml), — = no antibiotic resistance associated with these alleles.

^cData for *dam-3*, *dam-4* and *dam-13::Tn9* taken from Russel and Hirata (1989) nd = not detectable by the methods used, as described in the references.

TABLE II
Commonly used *dam* and *dcm* and wt strains

Strain ^a	Sex ^b	Pertinent ^c markers	Other markers ^d	Source or ^e Reference	Comments ^f
AB1157	F ⁻		<i>thr-1 ara-1 leuB6 lacY1 argE3</i> Δ (<i>gpt-proA</i>)62 <i>mtl-1 xyl-5</i> <i>rpsL31 tsx-33 supE44 galK2</i> <i>glnV44 hisG4(Oc) rfbD1</i> <i>kdgK51 rfb-1 mgI-51 thi-1</i> <i>thr-1 leuB6 lacY</i> <i>supE44 rfbD-1 thi-1</i> <i>tonA21</i>	CGSC	The prototype wt strain for many <i>dam</i> or <i>dcm</i> constructs.
C600	F ⁻		prototrophic	CGSC	A strain commonly used for gene cloning.
EMG2	F ⁺	λ		CGSC	The wt strain used to produce the first (<i>NotI</i>) macro-restriction map of the <i>E. coli</i> K-12 chromosome — [see legend to Fig. 1 and Smith et al. (1987)].
GM31	F ⁻	<i>dcm-6</i>	<i>thr-1 ara-14 leuB6 tonA31 lacY1</i> <i>tsx-78 glnV44 galK2 galT22 hisG4</i> <i>rpsL136 xyl-5 mtl-1 thi-1</i> <i>sup-85 (Am)</i>	Marinus (1973)	The prototype strain carrying <i>dcm-6</i> . The λ phages give good yields when propagated on this strain. Does not lack any restriction system.
GM33	F ⁻	<i>dam-3</i>		Marinus and Morris (1974)	A <i>dam-3</i> strain which grows very well and is useful for phage and plasmid propagation. Does not lack any restriction system.
GM48	F ⁻	<i>dam-3 dcm-6</i>	<i>thr-1 leuB6 ara-14 tonA31 lacY1 tsx-78 glnV44</i> <i>galK2 galT22 thi-1</i>	Marinus (1973)	The prototype <i>dam dcm</i> double mutant. Transformed at high efficiency by plasmid DNA. Does not lack any restriction system.
GM119	F ⁻	<i>dam-3 dcm-6</i>	<i>lacY1 galK2 galT22 tonA31</i> <i>tsx-78 supE44 mtl-1 (thi-1)[?]</i> <i>lacZ1118 Str^R</i>	M.G.M.	A prototrophic derivative of GM48.
GM124	F	<i>dam-4</i>		M.G.M.	A <i>dam-4</i> strain lacking amber suppressor mutations. Carries <i>lacZ1118</i> which allows for visual detection of <i>dam</i> deficiency. On lactose MacConkey medium forms white colonies with extensive red papillae after two days incubation at 37°C. Few papillae are formed in colonies of the wt strain. Good host strain for propagation of bacteriophages.
GM161	F	<i>dam-4</i>	<i>thr-1 leuB6 supE44 lacY1 tonA21</i> <i>hsdS1 (r_k⁻ m_k⁻)</i> <i>glnV44 (thi-1 rel-1)</i> <i>rns-1</i>	M.G.M.	A <i>dam-4</i> strain derived from C600 and lacking the K-12 R-M system.
GM215	F ⁻	<i>dam-3 end-1</i>		Arraj and Marinus (1983)	A <i>dam-3</i> strain lacking DNA endonuclease I and RNase I.
GM271	F	<i>dcm-6</i> <i>hsdR2</i>	<i>ara-14 leuB6 tonA31 lacY1 tsx-78</i> <i>glnV44 galK2 galT22</i> <i>hisG4 rpsL136 xyl-5 mtl-1 thi-1</i> <i>hsdS21 metB1 lacY or Z4 galK</i> <i>galT22 mtl-2 tonA2 or A31</i> <i>tsx-1 or 78 supE44 (thi-1)[?]</i>	M.G.M.	A <i>dcm-6</i> strain defective for K-12 restriction but not modification. Poorly transformed by plasmid DNA.
GM272	F	<i>dam-3 dcm-6</i>		M.G.M.	A derivative of GM119 which lacks the K-12 R-M systems. Has no detectable methylated bases in DNA.

TABLE II (continued)

Strain ^a	Sex ^b	Pertinent ^c markers	Other markers ^d	Source or Reference	Comments ^f
GM967	F ⁻	<i>hsdS</i>	<i>thi-1 leuB6 lacY1 supE44 tonA21 thi-1 rfbD1 metB1 Str^R Nal^R GM967</i>	M.G.M.	see section d (3)
GM1212	F ⁻	pN3	<i>A(lac-pro)km rxs-78</i>	M.G.M.	see section d (3)
GM1674	F ⁻	<i>F⁻lacI^a</i> <i>ΔM15 pro+ /</i> <i>dam-3 dcm-6</i>	<i>ghnV44 galK2 galT22 thi-1</i>	Arraj et al. (1990)	A <i>dam</i> <i>dam</i> F ⁻ strain suitable for propagation of M13 phages and plasmids which rely on β-galactosidase production by α-complementation. Contains all K-12 R-M systems but this is not a problem when propagating M13 phages since these ss phages are not subjected to K-12 restriction. As GM1674 but <i>dam</i> ⁻ .
GM1684	F ⁻	<i>F⁻lacI^a</i> <i>ΔM15 pro+ /</i> <i>dam-4</i>	<i>Δ(lac-pro)km thi-1 glnV44 (relA1)</i>	Arraj et al. (1990)	
GM1737	F	<i>dam-4</i> <i>cysG::Tn5</i>	<i>mut-354 rxs-354</i>	Arraj and Marinus (1983)	A <i>dam-4</i> strain with a linked <i>cysG::Tn5</i> marker which is cotransducible with <i>dam-4</i> using phage P1.
GM2159	F ⁻	<i>dam-13::Tn9</i> <i>recF143</i>	<i>thi-1 ara-14 leuB6 proA2 lacY1 glnV44 galK2 hisG4 rpsL31 xyl-5 mtl-1 argE3 thi-1 rxs-33</i>	Marinus et al. (1983)	A <i>dam-13::Tn9</i> strain which is deficient for plasmid recombination. Restriction proficient.
GM2163	F ⁻	<i>dam-13::Tn9</i> <i>dcm-6 hsdR2</i>	<i>leuB6 his-4 thi-1 ara-14 lacY1 galK2 galT22 xyl-5 mtl-1 rpsL136 tonA31 rxs-78 supE44</i>	M.G.M.	As GM2159 but lacking the <i>Mer</i> and <i>Hsd</i> restriction systems.
GM2198	F	<i>MerA⁻ MerB⁻</i> <i>dam-13::Tn9</i>	<i>thi-1 ara-14 leuB6 tonA31 lacY1 rxs-78 supE44 galK2 galT22 hisG4 rpsL136 xyl-5 mtl-1 thi-1</i>	M.G.M.	Together with GM30 (as GM31 but <i>dam</i> ⁺ and <i>dcm</i> ⁻) and GM31 (<i>dam-6</i>) these form an isogenic set of strains. GM 2198 is <i>dam-13::Tn9</i> and GM2199 is <i>dam-13::Tn9</i> and <i>dcm-6</i> . see comment for GM2198.
GM2199	F	<i>dam-13::Tn9</i> <i>dcm-6</i>	<i>thi-1 ara-14 leuB6 tonA31 lacY1 rxs-78 glnV44 galK2 galT22 hisG4 rpsL136 xyl-5 mtl-1 thi-1 lacI^a lacZpL8</i>	M.G.M.	
GM2290	F ⁻	pTP166 (<i>Dam</i> protein overproducer)	<i>thi-1 relA1</i>	Marinus et al. (1984)	A strain derived by transformation of W3110 with pTP166 (see section b for a description of this plasmid).
GM2807	Hfr (PO68)	<i>dam-16</i> (Δ: Km ^R)		M.G.M.	A <i>dam-16::Km^R</i> deletion mutant Hfr strain useful for strain construction and propagation of M13 phages.
GM2929	F ⁻	<i>dam-13::Tn9</i> <i>dcm-6 hsdR2</i> <i>recF143</i>	<i>galK2 galT22 ara-14 lacY1 xyl-5 thi-1 tonA31 rpsL136 hisG4 rxs-78 mtl-1 glnV44 leuB6 rfbD</i>	M.G.M.	This has become the standard <i>dam dcm</i> double mutant strain. It is deficient for plasmid recombination and lacks the <i>Mer</i> and <i>Hsd</i> restriction systems. The strain transforms well with plasmid DNA. Derived from GM2163.
GM2956	F ⁻	<i>MerA⁻ MerB⁻</i> as GM48 but	<i>hsdR17 thr⁻¹ leu⁺</i>	M.G.M.	A restriction deficient but modification proficient derivative of GM48.

GM3819	F ⁻	<i>dam</i> -16	<i>thr</i> -1 <i>leu</i> B6 <i>thi</i> -1 <i>arg</i> E3 <i>his</i> G4 <i>pro</i> A2 <i>lac</i> Y1 <i>gal</i> K2 <i>mtl</i> -1 <i>xyI</i> -5 <i>ara</i> -14 <i>rps</i> L31 <i>tsx</i> -33 <i>ghn</i> V44 <i>rfd</i> D1 <i>kds</i> GK51	Parker and Marinus (1988)	The prototype <i>dam</i> -16::K ^m R deletion strain.
GM4708	F ⁻	<i>dam</i> -16 <i>mut</i> D5	<i>thr</i> -1 <i>leu</i> B6 <i>thi</i> -1 <i>arg</i> E3 <i>his</i> G4 <i>lac</i> Y1 <i>gal</i> K2 <i>mtl</i> -1 <i>xyI</i> -5 <i>ara</i> -14 <i>rps</i> L31 <i>tsx</i> -33 <i>ghn</i> V44 <i>rfd</i> D1 <i>kds</i> GK51	Palmer and Marinus (1991)	A <i>dam</i> -16 <i>mut</i> D5 double mutant. This strain has a very high spontaneous mutation rate, see section f .
GM4715	F ⁻	<i>dam</i> -16	<i>trp</i> 31 <i>his</i> 1 <i>ton</i> A2 <i>rps</i> L104 Δ (<i>lacZ</i>) _{r1} <i>sup</i> E44 <i>xyI</i> -7 <i>mtl</i> -2 <i>met</i> R1 <i>mcr</i> A3 <i>arg</i> G6 Δ (<i>mcr</i> B- <i>hsd</i> - <i>mrr</i>) ₁₀ <i>hsd</i> R17 <i>sup</i> E44 <i>thi</i> -1 <i>leu</i> B6 <i>lac</i> Y1 <i>gal</i> K2 <i>gal</i> T22 <i>ara</i> -14 1 <i>ton</i> A3 <i>thr</i> -1 <i>tsx</i> -78 Δ (<i>lac</i> - <i>pro</i> A <i>B</i>) /F' <i>rad</i> 36 <i>lacZ</i> AM15 <i>lac</i> I ^a <i>pro</i> A <i>B</i> ⁺ IN(<i>rrnD</i> - <i>rrnE</i>)1 prototrophic	B.R.P., E. Raleigh and M.G.M.	<i>Ammr</i> <i>mcr</i> <i>dam</i> -16 mutant. Potentially useful as a host for cloning methylated DNA.
JM110	F ⁻	<i>dam</i> -3 <i>dcm</i> -6	<i>hsd</i> R17 <i>sup</i> E44 <i>thi</i> -1 <i>leu</i> B6 <i>lac</i> Y1 <i>gal</i> K2 <i>gal</i> T22 <i>ara</i> -14 1 <i>ton</i> A3 <i>thr</i> -1 <i>tsx</i> -78 Δ (<i>lac</i> - <i>pro</i> A <i>B</i>) /F' <i>rad</i> 36 <i>lacZ</i> AM15 <i>lac</i> I ^a <i>pro</i> A <i>B</i> ⁺ IN(<i>rrnD</i> - <i>rrnE</i>)1 prototrophic	Yanisch-Perron et al. (1985)	This strain is a derivative of GM48 modified for use with M13 and pUC vectors.
W3110	F ⁻			CGSC	The strain on which restriction map of Kohara et al. (1987) for <i>E. coli</i> K-12 is based.

^aFor a discussion of some of these strains' general features, see sections **f** and **g**

^bF⁻ plasmid carrier status.

^cThese markers describe the strain's *Dam* and/or *Dcm*, recombination and restriction status and also outline potential for *lacZ* α -complementation and in one instance a marker cotransducible with *dam*. The specific *dam* and *dcm* alleles are discussed in section **f**. Strains carrying PN3 require selection with Tc (10 μ g/ml) to ensure plasmid maintenance.

^dAll other markers excluding those outlined in footnote **c** above.

^eCGSC, Coli Genetic Stock Center, Yale University, New Haven CT 06510, USA (Curator Dr. B.J. Bachmann). Strains marked with initials are unpublished constructions.

^fFor a recent discussion of the structural differences in chromosomal structure of some of these strains, see Perkins et al. (1993).

lexA, *polA* and *ruv*. A *dam mutD5* double mutant, GM4708, has been shown to have an extremely high mutation rate in rich medium (up to approx. 10^4 greater than wt strains), presumably due to the combination of the loss of Dam-mediated mismatch repair strand discrimination and overloading of mismatch repair due to faulty proofreading. The spectrum of mutations produced in the reporter gene *mnt* in GM4708 more closely resembles a *dam*⁻ spectrum than a *mutD5* spectrum (Carraway et al., 1988; Palmer and Marinus, 1991; Wu et al., 1991).

(g) Description of *dam* and *dcm* strains

Table II gives a description of several of the most commonly used *dam*, *dcm* and wt strains. The *dam*-16 Hfr donor strain GM2807 is extremely useful for the construction of new *dam*-deficient strains. The *dam*-16 allele is transferred efficiently as an early marker, having a time-of-entry of approx. 6 min from this strain, and the desired recombinants can be easily isolated by a simple selection for Km^R in combination with a marker specific to the recipient strain (e.g., streptomycin resistance). Putative transconjugants can be tested for loss of Dam activity using the 2-AP test described above or by examining the restrictability of transconjugant DNA by *Mbo*I, *Dpn*I and *Sau*3AI. Alternatively, new *dam* strains can be constructed by P1 transduction and selection for the appropriate drug resistance (Km^R for *dam*-16 and Cm^R for *dam*-13).

(h) Plasmids containing the *dam* and *dcm* genes

A number of plasmids containing the *dam* gene have been constructed in an effort to study the gene and control its expression. Examples include:

pTP166, a pBR322 derivative with the *tac* promoter in front of the *dam* gene and two endogenous promoters (Marinus et al., 1984).

pMQ191, as above but in a pACYC184 backbone.

pALO160, a single copy *dam* plasmid with an R1 origin (Løbner-Olesen et al., 1992).

pYin4, a ColE1-derived plasmid with the *dam* gene under control of the *p_l* promoter of phage λ (Nwosu, 1992).

Plasmids containing the *dcm* gene have been used to determine the gene's nt sequence and study the *vsr* gene. An example is:

pDCM1, a pBR322 derivative containing the *dcm* gene on an 11-kb fragment cloned into the *Bam*HI site (Bhagwat et al., 1986).

(i) Sources

A comprehensive range of *dam* and *dcm* *E. coli* strains is available for use for molecular manipulation, DNA

cloning and for the study of bacterial DNA methylation. The strains described here are available free of charge from the laboratory of M.G.M. Some of these strains are also available from the Coli Genetic Stock Center, Yale University, New Haven CT 06510, USA. A variety of *dam* and *dcm* strains are also available from commercial sources.

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