

Location of DNA Methylation Genes on the *Escherichia coli* K-12 Genetic Map

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Summary. The genes responsible for DNA adenine methylation (*dam*) and DNA cytosine methylation (*dcm*) have been mapped on the *E. coli* K-12 genetic map. The *dam* gene is situated at min 65 and the gene order *cysG*-(*trpS*,*dam*)-*aroB* inferred. The *dcm* gene is located at min 37.5 and the gene order is *supD*-*dcm*-*flaA1*. In F' merodiploids, the *dam* and *dcm* alleles are recessive.

1. Introduction

Although methylated bases in DNA are widespread throughout nature (Hall, 1971) the biological role of such bases is for the most part unknown. It has been established in bacteria, however, that a minute fraction of the total N6-methyladenine (6-MeA) is involved in host-specific modification of DNA (Smith *et al.*, 1971). There is also evidence that 5-methylcytosine (5-MeC) residues play a role in host-specific modification (Hattman *et al.*, 1972). As a first step to define the role of methylated bases in DNA, Marinus and Morris (1973) isolated mutants of *Escherichia coli* K-12 in which DNA was undermethylated. One class of mutants (*dcm*) was shown to be devoid of 5-MeC while another class had reduced amounts of 6-MeA (*dam* mutants). 5-MeC and 6-MeA are the only detectible methylated bases in the DNA of *E. coli* K-12.

It was expected that any difference in cellular function between the methylation deficient mutants and their wild type would be due specifically to the *dam* or *dcm* mutations. These mutations, however, were induced by nitrosoguanidine which produces more than one mutation per chromosome (Guerola *et al.*, 1971). To minimize the effects of extraneous mutations in detailed comparative studies between methylation mutants and wild type, it has been necessary to map the *dam* and *dcm* mutations and transduce them into another genetic background. These genetic studies are reported in this communication.

2. Materials and Methods

a) Bacterial Strains

Donor Strains. The Hfr strains used are described in Table 1 and most were obtained from the *E. coli* Genetic Stock Center (CGSC), Department of Microbiology, Yale University, New Haven, Connecticut, U.S.A. The point of origin of each Hfr strain is shown in Fig. 1. The chromosomal regions carried by the F' elements F131, F141, F148, and F4102 are shown in Figs. 1 and 2. *Recipient strains.* These are described in Table 1.

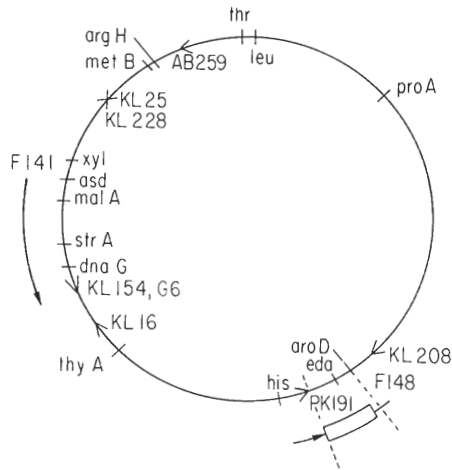


Fig. 1. Map of *E. coli* K-12 showing: pertinent auxotrophic markers (After Taylor and Trotter, 1972); the transfer origins of the Hfr strains used and the chromosomal regions covered by F141 and F148. The rectangle in F148 denotes a deletion

Table 1. Genotypes of the strains used in this study

Strain	Sex	Pertinent genotype	Other markers	Source
AB259	Hfr		<i>thi-1</i> ^a <i>rel-1</i>	CGSC ^b
AT2455	Hfr	<i>cysG 44</i>	<i>thi-1 rel-1, mal-18</i>	CGSC
KL16	Hfr		<i>thi-1 rel-1</i>	CGSC
KL25	Hfr		<i>sup-42</i>	CGSC
KL154	Hfr		<i>purF mtl-2 xyl-7 gal-6 lacY1</i> or <i>Z4</i>	B. Low
KL208	Hfr		<i>rel-1?</i>	CGSC
KL228	Hfr		<i>thi-1 leu-6 gal-6 sup54 lacY1</i> or <i>Z4</i>	CGSC
G6 MD2	Hfr	Δ (<i>bioH-mal-asd</i>)	<i>his</i>	M. Schwartz
GM42	Hfr	<i>dam-3</i>	<i>his</i>	A ^c
PK191	Hfr		<i>thi-1</i> Δ (<i>proB-lac</i>) _{XII} <i>sup-56</i>	CGSC
F131/ JC1553	F'	<i>F-his⁺-supD</i>	<i>metB1 leu-6 his-1 argG6 lacY1</i> or <i>Z4 malA1 tonA2 xyl-7 mtl-2 gal-6 str-6 str-104 recA1</i>	B. Low

^a Symbols used: *ara*, arabinose; *aro*, aromatic; *asd*, aspartate semialdehyde dehydrogenase; *che*, chemotaxis; *cys*, cysteine; *dam*, DNA adenine methylation; *dcm*, DNA cytosine methylation; *dna*, DNA replication defective; *dra*, deoxyriboaldolase; *eda*, Entner-Doudoroff aldolase; *fla*, flagella; *gal*, galactose; *gnd*, gluconate-6-phosphate dehydrogenase; *hag*, H antigen; *his*, histidine; *lac*, lactose; *leu*, leucine; *mal*, maltose; *mec*, 5-methylcytosine in DNA; *mtl*, manitol; *met*, methionine; *mot*, motility; *pro*, proline; *pur*, purine; *rel*, relaxed RNA synthesis; *str*, streptomycin; *sup*, suppressor; *this*, thiamine; *thr*, threonine; *thy*, thymine; *ton*, T-one phage; *trp*, tryptophane; *tsx*, T-six phage; *uvr*, ultraviolet irradiation sensitive; *xyl*, xylose.

^b CGSC = Coli Genetic Stock Center.

^c A = This laboratory.

Table 1 (continued)

Strain	Sex	Pertinant genotype	Other markers	Source
F141/ JC1553	F'	F- <i>argG</i> ⁺ - <i>asd</i> ⁺	As F131/JC1553	GCSC
F148/ KL159	F'	F- <i>his</i> ⁺ - Δ (<i>che-C-argS</i> ?) <i>aroD</i> ⁺ - <i>aroH</i> ⁺	<i>his aroD recA str thi pro xyl? lac</i>	B. Low
F4102	F'	F- <i>his</i> ⁺ - <i>wvrC</i> ⁺	<i>recA nalA thyA</i> Δ (<i>his-gnd</i>) <i>trpA galE</i>	J. S. Parkinson
AB2847	F ⁺ ?	<i>aroB351</i>	<i>mal-354 tsx-354</i>	CGSC
AB2848	F ⁺	<i>aroD352</i>	<i>thi-22 tsx-354</i>	CGSC
AB3640	F ⁻	<i>dnaG308</i>	<i>thi-1 thr-1 leu-6 lac Y1 str-67</i> <i>dra-8 thyA6</i>	CGSC
GM1	F ⁻		<i>thr-1 leu-6 thi-1 lac Y1 galK2</i> <i>ara-14 xyl-5 mtl-1 proA2</i> <i>his-4 metB1 str-31 tsx-33</i> <i>sup-37 thyA12 thyR14</i>	A
GM2	F ⁻	<i>dam-1</i>	As GM1	A
GM6	F ⁻	<i>dcm-2</i>	As GM1	A
GM10	F ⁻	<i>dcm-6</i>	As GM1	A
GM11	F ⁻	<i>dcm-7</i>	As GM1	A
GM31	F ⁻	<i>dcm-6</i>	<i>gal ara lac xyl thr leu thi</i> <i>tonA tsx str</i>	A
GM48	F ⁻	<i>dam-3 dcm-6</i>	<i>gal ara lac thr leu thi? tonA tsx</i>	A
Hag	F ⁻	<i>hag-585</i>	<i>gal ara lac xyl thr leu thi</i> <i>tonA str his tsx</i>	J. Adler
MS371	F ⁻	<i>flaA1, hag-207</i>	<i>wvrC34 galU str-31 his-4 arg E3 thy</i>	M. Silverman
RP463	F ⁻	<i>eda</i>	<i>thi thr leu lac ara xyl mtl</i> <i>str tonA tsx</i>	J. S. Parkinson
RP3326	F ⁻	<i>mot-526</i>	<i>thi thr leu his lac ara xyl mtl</i> <i>gal str tonA tsx</i>	J. Adler
TrpS	F ⁻	<i>trpS10330</i>		C. Yanofsky

b) Media

Complete medium was Difco brain heart infusion broth (20g/L) solidified, when required, with 1.6% Difco agar. The minimal medium was that of Davis and Mingioli (1951). Motility agar was that described by Armstrong and Adler (1969).

c) Conjugation Procedure

The procedure of Adelberg and Burns (1960) was used. Donor and recipient strains were mated at a ratio of 1:20 for 60 min at 37C after which the culture was blended for 1 min, diluted and plated in soft agar overlays on selective media.

d) Cross-streak Test for Transfer of F' markers

F' merodiploids were cross-streaked against F⁻ strains on selective media. Confluent growth of recombinants was obtained in the cross-streak area if the selected marker was located

on the episome, whereas only a few recombinant colonies were obtained if selection was made for chromosomal markers.

e) Preparation of Transducing Lysates and Transduction Procedure

Pl *vir* lysates were prepared in liquid medium and used to transduce recipient strains as described by Ikeda and Tomizawa (1965).

f) Selection of Recombinants

For markers having auxotrophic requirements (e, g., *his*), selection was made for prototrophic recombinants (i.e., His⁺). *Eda*⁺ selection was accomplished by including glucuronic acid as the sole carbon source in minimal medium. Hag⁺, FlaA⁺ and Mot⁺ clones were those that swarmed on motility agar. Asd⁺ recombinants were those able to grow on brain heart infusion agar. Dna B⁺ recombinants as those able to grow at 43 C, *dam* and *dcm* were scored as described in section (h). All recombinant clones were purified twice by streaking single colonies on selective media before being scored.

g) Preparation of Crude Extract

Late log phase cultures of GM1 grown in broth at 30C were harvested and washed twice with 0.01 M Tris-0.001 M EDTA, pH 7.8. The cells were ground with twice their wet weight of alumina and extracted with five volumes of 0.01 M Tris-0.001 M dithiothreitol-10% glycerol, pH 7.8. After removal of the alumina by centrifugation, MgSO₄ was added to 1 mM and the extract incubated for 30 min at room temperature to hydrolyse endogenous DNA. After incubation, EDTA was added to a final concentration of 2 mM. The crude extract was clarified by centrifugation at 12000 × g for 20 min at 4C, dispensed into tubes and stored frozen at -60C.

h) Scoring of Recombinants for *dam* and *dcm*

Purified recombinants were each inoculated into 20 ml complete medium and grown for 16 hrs at 32C. Total nucleic acids were isolated from each culture by lysing the washed cells with sodium lauryl sulfate at 60C (Marmur, 1961). The mixture was deproteinized with chloroform: isoamyl alcohol (24:1) and the nucleic acids precipitated from the aqueous phase with ethanol (70% final concentration). The nucleic acid precipitate was washed once with 95% ethanol and once with ether. To the substrate DNA was added: crude extract, 250 µg-lmg protein; 0.04 M Tris-0.001 M EDTA, pH 7.8; 6.3 × 10⁻⁶ M (³H-methyl)-S-adenosyl-L-methionine (379 Ci/M) and water to a final volume of 250 µL. The reaction time was 30 min at 37C. The reaction was stopped by the addition of 0.2 ml 25% sodium lauryl sulfate, 500 µg salmon sperm DNA as carrier, water to a final volume of 2 ml followed by heating at 60C for 10 min. Sodium perchlorate was added to a final concentration of 1 M and the mixture shaken with an equal volume of chloroform: isoamyl alcohol. The aqueous phase was removed after centrifugation, NaOH added to 0.5 M and the mixture heated for 10 min at 60C to hydrolyse RNA. After cooling, the solution was precipitated with 0.5 M perchloric acid and washed three times with this acid. The precipitate was resuspended in 0.5 M perchloric acid and heated at 90C for 10 min, after which it was dissolved in scintillation fluor which consisted of 4 gm "Omni-fluor" (New England Nuclear), 667 ml toluene and 333 ml absolute alcohol. The DNA from wild type strains gave 50-80 counts/min and DNA from *dam* or *dcm* strains 500-1000 counts/min.

3. Results

a) Scoring of *dam* and *dcm* Recombinants

The methylation mutants differ from their wild type in that their DNA is under-methylated. They can be recognized by the use of an *in vitro* assay (Marinus and Morris, 1973) in which wild type DNA methylases are reacted with substrate DNA and the extent of methyl group transfer determined. A modified version of this

Table 2. Linkage of *dam* and *dcm* to other markers in Hfr \times F⁻ crosses

Donor	Recipient	Selected marker(s)	Unselected marker)	Linkage (%)
KL25	GM2	Thr ⁺ Leu ⁺	<i>dam-1</i>	0
KL228	GM2	Xyl ⁺ Str ⁺	<i>dam-1</i>	40
KL154	GM2	Met ⁺	<i>dam-1</i>	60
AB259	GM10	His ⁺	<i>dcm-6</i>	90
KL16	GM10	His ⁺	<i>dcm-6</i>	50
PK191	GM10	His ⁺	<i>dcm-6</i>	0
KL208	GM10	Pro ⁺	<i>dcm-6</i>	0

Donor and recipient cells were mated at a ratio of 1:20 for 60 min at 37C after which the cultures were blended for 1 min, diluted and plated in soft agar overlays on selective media.

assay, described in Methods and Materials, has been used in the scoring of recombinants arising from crosses to be described because no phenotypic trait has yet been discovered which allows for the direct selection of the *dam* or *dcm* phenotype or which allows such mutants to be recognized easily. Consequently, the methylation mutations could be scored only as unselected markers in genetic crosses. Furthermore, only ten recombinants from each cross were analyzed for their ability to accept methyl groups into DNA.

Two strains, GM2 (*dam-1*) and GM10 (*dcm-6*) were used as examples to determine the positions of the *dcm* and *dam* genes on the chromosome of *E. coli*.

b) Hfr Crosses

Strains GM2 (*dam-1*) and GM10 (*dcm-6*) were each mated with various Hfr donors to determine which of these injected the *dam*⁺ or *dcm*⁺ as early markers. The results are given in Table 2. When GM10 was mated with AB259 or KL16 and His⁺ recombinants selected, 90% and 50% respectively of these recombinants had also inherited the *dcm*⁺ allele. The results indicate that *dcm* is close and counterclockwise to *his*. Neither KL208 or PK191 transfer *dcm*⁺ as an early marker, indicating that the gene lies between the origins of these Hfr strains (30–37.5 min).

Similarly, KL154 and KL228 transfer the *dam*⁺ allele as an early marker (Table 2). Since *dam-3* was present in 40% of the Xyl⁺ Str^r recombinants derived from the KL228 \times GM2 cross, this marker lies clockwise to *strA*. This was confirmed by analyzing the distribution of unselected markers among the Met⁺ recombinants arising from the KL154 \times GM2 cross. In every case *dam*⁺ recombinants were Str^r and *dam* recombinants were Str^s. The *dam* mutation is situated, therefore between *strA* and the origin of KL228 (minute 72).

c) F' Cross

Various F' factors were introduced into GM2 or GM10 to determine if the methylation mutations were recessive or dominant to their wild type alleles and to determine more precisely the position of the methylation mutations.

The DNA of F148/GM10 merodiploids was undermethylated, whereas the DNA of F4102/GM10 and F131/GM10 merodiploids was fully methylated.

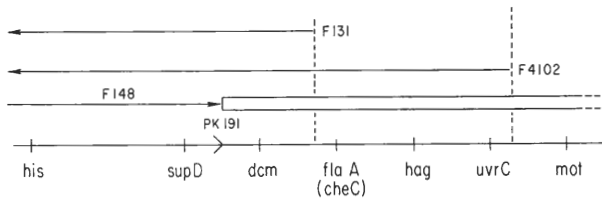


Fig. 2. The location of *dcm* in relation to nearby markers. The figure shows the point of origin of Hfr PK 191 and the chromosomal regions covered by F 131, F 148 and F 4102. The rectangle in F 148 denotes a deletion

Table 3. Linkage of *dam* and *dcm* to other markers in transductional crosses

Selected marker(s)	Unselected marker	Linkage (%)
TrpS ⁺	<i>dam-1</i>	90
MalA ⁺	<i>dam-1</i>	10
AroB ⁺	<i>dam-1</i>	80
CysG ⁺	<i>dam-1</i>	50
StrA ⁺	<i>dam-1</i>	10
Asd ⁺	<i>dam-1</i>	5
Eda ⁺	<i>dcm-6</i>	0
Mot ⁺	<i>dcm-6</i>	10
Hag ⁺	<i>dcm-6</i>	10
FlaA1-Hag ⁺	<i>dcm-6</i>	30

Pl *vir* lysates of *dam-1* or *dcm-6* strains were incubated with recipients at a multiplicity of 0.05. After 20 min at 37°C, the mixture was plated on selective media. 20 Asd⁺ recombinants were analysed for *dam*.

These data indicate that *dcm-6* is situated between the origin of F 148 and the terminus of F 131 (Fig. 2). The identity of the merodiploids was confirmed by two methods. First, in genetic crosses with marked F⁻ strains, episomal markers were transferred at high frequency but chromosomal markers were not. Second, His⁻ segregants were isolated from the merodiploids after growth in broth. These segregants were *dcm* and unable to transfer episomal markers. They were also able to act as recipient when mated with F' donors. The *dcm-6* allele is, therefore, recessive to its wild type allele.

The DNA of F 141/GM2 merodiploids was fully methylated. The *dam-1* mutation must lie between *strA* and the terminus of F 141 (minute 66). F 141/GM2 merodiploids transferred DnaG⁺ and MalA⁺ at high frequency but not ArgH⁺. They were also Str^s. Ten Str^r segregants were isolated and were unable to transfer DnaG⁺, MalA⁺, or ArgH⁺. Five of these segregants were *dam*. The *dam-1* gene is, therefore, recessive to its wild type allele.

d) Transductional Crosses

Pl *vir* lysates were prepared from GM2 and GM10 and used to transduce various mutant alleles in the 64–66 min and 36–37 min regions of the chromosome

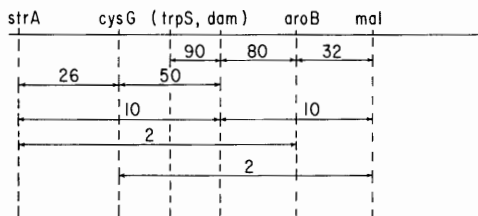


Fig. 3. The location of *dam* in relation to nearby markers. The figures denote the percentage co-transduction between pairs of markers. Ten recombinants were analysed in crosses involving *dam* and 100 recombinants were analysed in other crosses

to prototrophy. The results are given in Table 3. The *dcm-6* gene was co-transducible with *hag-585* and *mot-526* but not *eda*. When selection was made for $\text{FlaA1}^+\text{-Hag}^+$, 30% of the transductants were *dcm*. This result implies that the gene order is *dcm-flaA1-hag* (Fig. 2). It has recently been demonstrated that *flaA1* and *cheC* are mutant in the same cistron (M. Silverman, *personal communication*). Strains bearing *flaA* or *cheC* have fully methylated DNA as does Hfr PK 191.

The *dam-1* gene is co-transducible with *cysG*, *trpS*, *malA*, *asd* and *strA* (Table 3). The data are insufficient to allow *dam* to be placed either to the right or left of *trpS* (Fig. 3). A three factor cross is needed to determine the order of markers but this is not possible at this time since *dam* recombinants can not be distinguished from the wild type. During the mapping of *dam* by transduction, linkage data was also obtained for other markers in the *strA-malA* region. These are given in Fig. 3.

e) Location of Other *dam* and *dcm* Mutations

Three *dam* mutants were isolated by Marinus and Morris (1973). The *dam-3* gene is also 90% co-transducible with *trpS* and it is probably mutant in the same gene as *dam-1*. The strain carrying *dam-2* is unable to grow on minimal medium and is also resistant to Pl *vir*. Consequently, no mapping studies have been done with this strain.

All the *dcm* mutants were shown to be *dcm*⁺ after infection with F4102 except. GM11 (*dcm-7*) and GM6 (*dcm-2*) which were not tested since they are unable to grow on minimal medium. The *dcm-2* and *dcm-7* mutations, however, were 25% (2/8) cotransducible with *flaA1-hag*. All the *dcm* mutations tested, therefore, map in the same small region of the chromosome.

f) Construction of a *dam dcm* Mutant

A *dam-3* transductant, GM42, was isolated by selection for Asd^+ . This strain is derived from HfrG6 which has its point of origin at 60 min and transfers its DNA in a counterclockwise direction (Fig. 1). Strain GM42 was mated with GM31 (a Hag^+ transductant) and selection made for Xyl^+Str^s recombinants. 80% of the recombinants were *dam-3*. The DNA of one of the recombinants (GM48) was analyzed by *in vitro* methylation and shown to be deficient in both 5-MeC and 6-MeA. That the strain is viable shows that loss of 5-MeC residues and most of the 6-MeA residues in DNA is not lethal.

4. Discussion

The combined use of conjugational and transductional crosses has allowed the mapping of *dam* and *dcm* mutations, in spite of the fact that only ten recombinants from each cross were analyzed. The genetic analyses are necessarily crude since there is no obvious phenotype associated with *dam* or *dcm* which enables these markers to be selected for directly or to be easily recognized. This will hamper fine structure analysis and has prevented the selection of revertants for these loci. It should be possible, however, to do complementation tests providing that *dam* and *dcm* F-prime elements can be constructed.

The data from Table 2, F' crosses and Table 3 show that the *dcm* gene is located between the origin of Hfr PK 191 and *flaA1* (Fig. 2). This result agrees with the approximate location proposed by Mamelak and Boyer (1970), who found that it was closely linked to *his* in intergeneric crosses between *E. coli* K-12 and B. It should be noted that the gene symbols *mec* (5-methylcytosine in DNA; Mamelak and Boyer, 1970; Taylor and Trotter, 1972) and *dcm* (DNA cytosine methylation) are synonymous. The designation *dcm* has been used in this laboratory to avoid confusion with another mutant which has no 5-MeC in RNA.

The *dam* gene is located near min 65 and is co-transducible with several markers in this region (Table 3; Fig. 3). In the absence of three factor cross data, it was not possible to determine equivocally the gene order. It is quite clear, however, that *dam* is well separated from the *hsp* genes which specify DNA adenine modification methylase (Boyer, 1970; Messelson, Yuan and Haywood, 1971). The residual adenine methylation (17-43%) observed in the *dam* (Hsp⁺) mutants *in vivo* (Marinus and Morris, 1973), may be due to the *hsp* gene product. It should now be possible to construct a *dam hsp* strain to test this possibility.

The finding that both *dam* and *dcm* are recessive to their wild type alleles, rules out the possibility that these genes are producing an inhibitor of DNA methylation such as that described by Falaschi and Kornberg (1965). If *dam* and *dcm* were producing DNA methylase inhibitors they would be dominant in merodiploids.

The purpose of isolating DNA methylation mutants was to understand the function of methylated bases in DNA. With the *dam* and *dcm* mutations in "clean" genetic backgrounds, differences between wild type and mutants in cellular functions are now being sought.

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