

PLEIOTROPIC EFFECTS OF A DNA ADENINE METHYLATION MUTATION (*dam-3*) IN *ESCHERICHIA COLI* K12

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SUMMARY

The *dam-3* mutation results in a five-fold reduction in the number of 6-methyladenine (6-meA) residues in the DNA of *E. coli* K12 or phage λ . The DNA of phage λ appears to be devoid of 6-meA when propagated on *dam-3* bacteria. The phenotypic differences between *dam-3* and *dam*⁺ bacteria include: (1) increased free phage in lysogenic *dam-3* cultures, (2) increased sensitivity to methyl methanesulfonate (MMS), (3) inviability of *dam-3 lex-1* strains, (4) lower molecular weight of DNA in *dam-3* bacteria in the absence of DNA ligase and (5) increased rate of DNA degradation in *dam-3 recA* strains.

INTRODUCTION

The DNA of *Escherichia coli* K12 contains 0.38 mole per cent 6-meA. It has been established that a small fraction of the total 6-meA^{1,10,16,19} confers protection against endonucleolytic cleavage by the K12 restriction enzyme, endonuclease III^{5,21}. The 6-meA residues that are involved in restriction-modification are the product of methylation by a DNA adenine methylase produced by the *hsp* gene cluster^{5,21}.

The major portion of the 6-meA residues in *E. coli* DNA appears to be the product of methylation by a DNA adenine methylase specified by the *dam* gene, since *dam* mutants have reduced amounts of adenine methylase activity *in vitro* and *in vivo*¹⁹. The *dam* mutants, however, do contain normal amounts of *hsp* gene product and can modify foreign DNA as efficiently as *dam*⁺ bacteria. Strains mutant at the *dam-3* locus differ from *dam*⁺ cells in (i) an increased mass per cell, (ii) increased sensitivity to UV-irradiation, (iii) increased generalized spontaneous mutability and (iv) that *dam-3* in combination with *recA* or *recB* or *recC* or *polA* results in cell death. Furthermore, the *dam-3* bacteria have chromosomal breaks in DNA and the number of breaks is increased in the absence of DNA polymerase I or DNA ligase²⁰. These data suggest that a biological function of 6-meA may be to protect DNA from degradation. In the present communication, additional data are presented to support this hypothesis.

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Abbreviations: *endA*, endonuclease I; 6-meA, N⁶-methyladenine; 5-meC, 5-methylcytosine; MMS, methyl methanesulfonate.

MATERIAL AND METHODS

(a) Bacterial strains and bacteriophages

The genotypes of the bacterial strains used in this study are listed in Table I. Phages T3 and T7 were obtained from W. McAllister; T4 and λ from S. Champe; Φ X174 \dagger B from D. R. Bone; T6 from D. Vapnek; fd from S. Hattman; λ CI₈₅₇S₇ from V. Bryson; λ CI₈₅₇gam-210 from A. Skalka; T3 sam⁻ from R. Hausmann; λ red-3 from C. Radding; T4amH39X and T4am605 from J. Karam.

TABLE I

GENOTYPE AND DERIVATION OF *E. coli* K12 STRAINS

Strain	Sex	Genotype ^a	Derivation
GM42	Hfr	<i>dam-3 hisA323</i>	A ^b
GM81	Hfr	<i>purF1 metB1 gal-6 lacY1</i> or <i>Z4 tonA2 tsx-1 dam-3</i>	GM42 × JC12
JC12	Hfr	As GM81 but <i>dam</i> ⁺ <i>mtl-2 xyl-7</i>	K. B. Low
N2672	Hfr	<i>lig-7</i>	M. M. GOTTESMAN
F42/GM31	F'	<i>dcm-6 thr leu thi tonA his str^R gal ara lac xyl tsx</i> F- <i>lac</i>	A
F42/GM48	F'	As F42/GM31 but <i>dam-3 xyl</i> ⁺ <i>str^S</i>	A
AB1884	F ⁻	<i>wvrC34 thi-1 argE3 his-4 proA2 leu-6 thr-1 mtl-1 xyl-5 ara-14 galK2 lacY1 tsx-33 str-31 supE44</i>	CGSC ^c
AB2500	F ⁻	As AB1884 but <i>wvrA6 thyA15 drm-2 wvrC</i> ⁺	CGSC
AB3642	F ⁻	<i>thr-1 leu-6 thi-1 lacY1 tonA2 str-67 thyA6 dra-1 supE44</i>	CGSC
DM803	F ⁻	<i>metA28 lac-24 thi-1 xyl-5</i> or <i>-7 galK2 tsx-6 lex-1</i>	K. B. Low
GM28	F ⁻	Prototrophic, W3110	A
GM33	F ⁻	As GM28 but <i>dam-3</i>	A
GM44	F ⁻	<i>thr-1 leu-6 proA2 metB1 lacY1 galK2 ara-14 tsx-33 thi-1 thyA21 thyR14 supE44</i>	GM42 × GM1
GM45	F ⁻	As GM44 but <i>dam-3</i>	GM42 × GM1
GM55	F ⁻	As AB2500 but <i>dam-3 xyl</i> ⁺ <i>str^S his</i> ⁺	GM42 × AB2500
GM66	F ⁻	As GM45 but <i>thyA</i> ⁺ <i>lig-7</i>	N2672 × GM45
GM67	F ⁻	<i>lac-44 thi-1 galK2 tsx-6</i>	GM42 × DM803
GM68	F ⁻	As GM67 but <i>dam-3</i>	GM42 × DM803
GM69	F ⁻	<i>dnaB43 lacZ608 lacI3 his-4 thyA21 thyR14 xyl-5 str-31 thi-1 mtl-1</i>	A
GM73	F ⁻	As AB1884 but <i>dam-3 xyl</i> ⁺ <i>str^S</i>	GM42 × AB1884
GM75	F ⁻	<i>dam-3 dnaB43 thi-1 leu-6 proA2 lacY1 galK2 ara-14 tsx-33 thyA21 thyR14 supE44 thi-1 str^R mtl-1</i>	A
GM79	F ⁻	As GM66 but <i>dnaB43 met</i> ⁺	A
GM83	F ⁻	As GM75 but <i>dam</i> ⁺ <i>his-4 thyA</i> ⁺ <i>lig-7 argE3</i>	A
GM84	F ⁻	As KL399 but <i>dam-3 xyl</i> ⁺ <i>str^S</i>	GM42 × KL399
GM85	F ⁻	As GM69 but <i>dam-3 xyl</i> ⁺ <i>mtl</i> ⁺ <i>str^S</i>	GM42 × GM69
KL399	F ⁻	<i>recA200 leu-6 proC32 metE70 hisF860 thyA thi-1 xyl-5 str-109 (ara-14 lacZ36 mtl-1 tsx^R azi^R tonA^R)</i> ?	K. B. Low

^a For explanation of genetic symbols, see TAYLOR AND TROTTER²⁷ and MARINUS¹⁷.

^b A, this laboratory.

^c CGSC, *E. coli* Genetic Stock Center, Department of Human Genetics, Yale University, New Haven, Conn. 06510 (U.S.A.).

(b) Media

The minimal medium was that of DAVIS AND MINGIOLI⁸. K medium is minimal medium supplemented with 1% Difco casamino acids. Complete medium is Difco brain heart infusion broth (20 g/l) solidified, when required, with 1.6% Difco agar. L broth consists of 10 g tryptone, 5 g yeast extract and 5 g NaCl per l. λ agar was described previously²⁰. Soft agar consists of 0.6% agar.

(c) *Assay for free phage*

The phage indicator strain was AB3642 which was prepared as described elsewhere²⁰. λ lysogens were cultured in L broth and 1-ml aliquots were removed, chloroformed and portions incubated with the indicator strain AB3642 for 20 min at 37°, after which the mixture was plated in soft agar overlays on λ agar containing 100 μ g/ml streptomycin.

(d) *UV-Irradiation*

Stationary-phase cultures were diluted in cold minimal medium to give $5-10 \cdot 10^6$ cells/ml. 3-ml portions in 10-cm petri dishes were irradiated at a distance of 43 cm or 100 cm from a General Electric Germicidal lamp. Survival was determined by plating dilutions on brain heart infusion agar and incubating in the dark at 37° for 48 h. The rate of UV-induced mutagenesis was determined by irradiating as above and plating on lactose minimal medium. The plates were scored after 4 days' incubation at 37°.

(e) *Methyl methanesulfonate (MMS) treatment*

Bacterial strains in the logarithmic phase of growth were diluted into minimal medium containing 0.15 M NaCl and 0.05 M MMS at 37° to a final cell concentration of $1-2 \cdot 10^6$ /ml. Samples were removed at intervals, diluted and plated on brain heart agar. The plates were scored after 24-h incubation at 37°.

(f) *Measurement of post-replication repair*

The method described by RUPP AND HOWARD-FLANDERS²⁵ was used. Exponentially growing cultures of bacteria were irradiated for 15 sec and incubated in K medium for 10 min at which time $1 \cdot 10^{-6}$ M [³H]thymidine (50 Ci/mmmole) was added. 10 min later, non-radioactive thymidine was added to $1 \cdot 10^{-4}$ M, the culture harvested and divided in two parts. The bacteria in one part were lysed immediately and the remainder was incubated for 70 min in the dark before harvesting and subsequent lysis. Portions of the lysates were sedimented in alkaline sucrose as described below.

(g) *Genetic procedures*

The methods for conjugation and transduction have been described elsewhere¹⁷.

(h) *In vivo assay of methyl group incorporation into DNA*

Bacteria were grown in minimal medium at 37° to $1-2 \cdot 10^8$ /ml and either $2.7 \cdot 10^{-3}$ M [L-¹⁴C]methionine (18 Ci/mmmole) or $3 \cdot 10^{-4}$ M [¹⁴C]adenine (33 Ci/mmmole) was added. 2 h later the cells were harvested, the DNA extracted, purified, hydrolysed and the bases chromatographed as described previously¹⁹. Phage λ DNA was extracted from radioactively labeled induced lysogens as described by BOVRE AND SZYBALSKI⁴. Phage fd DNA was prepared as described by HATTMAN¹¹.

(i) *Labeling conditions and sedimentation of DNA in sucrose gradients*

Bacteria were grown in K medium with $1 \cdot 10^{-5}$ M [¹⁴C]thymidine (50 mCi/mmmole) at 30°. For *thyA*⁺ strains 200 μ g deoxyadenosine/ml was included in the medium. At a cell concentration of $1-2 \cdot 10^8$ /ml the cells were harvested and lysed with lysozyme, EDTA and Sarkosyl²⁹. Portions of the lysate, together with radioactively labeled λ or T₄ DNA were sedimented in 5-20% linear sucrose gradients in an SW39 rotor at

30000 rev./min at 20° for times indicated in the text. Alkaline gradients contained 0.3 M NaOH, 0.7 M NaCl and 0.001 M EDTA. Neutral gradients contained 0.01 M Tris-HCl, pH 7.8, 0.001 M EDTA and 1.0 M NaCl. The gradients were collected by pumping from the bottom of the tube and five-drop fractions were collected on Whatman 3MM filter paper discs which were processed as described previously¹⁸. Recovery of input DNA was greater than 90%. The equation $S_1/S_2 = (M_1/M_2)^{0.38}$ and a molecular weight of $16 \cdot 10^6$ and $55 \cdot 10^6$ for single-stranded λ and T4 DNA respectively were used to calculate the molecular weight of DNA.

(f) *Release of radioactive thymidine from labeled DNA*

The release of radioactive thymidine from labeled DNA was measured as described by CLARK *et al.*⁷.

RESULTS

(a) *Adenine methylation of bacterial and phage DNA in dam⁺ and dam-3 bacteria*

Strains of bacteria mutant at the *dam-3* site are defective in methylating adenine residues in DNA but methylate cytosine residues in DNA to the same extent as *dam⁺* bacteria¹⁹. Two methods have been used to determine the mole per cent 6-meA in DNA. The first method involves labeling the DNA with radioactive adenine and determining the amount of label in adenine and 6-meA. The data in Table II show that the mole per cent 6-meA in *dam⁺* and *dam-3* bacteria is 0.38 and 0.04 respectively. The second method utilizes the fact that the methyl groups in 6-meA or 5-meC are derived from L-methionine. When *dam⁺* or *dam-3* strains are grown in the presence of radioactive L-methionine, the ratio of radioactive label in 6-meA to 5-meC in *dam⁺* and *dam-3* bacteria is 1.7 and 0.24 respectively (Table II). From these ratios the mole per cent 6-meA in *dam⁺* and *dam-3* bacteria has been calculated to be 0.38 and 0.06 respectively. The results from the two methods are quite comparable.

Table II also shows that the DNA of phage λ contains less 6-meA when propagated in a *dam-3* host as compared to a *dam⁺* host and the extent of adenine undermethylation is the same as for the host bacteria. The amount of 6-meA in phage fd has also been deter-

TABLE II

In vivo ADENINE METHYLATION OF BACTERIAL AND PHAGE DNA

In vivo incorporation of methyl groups into DNA was determined as described in MATERIALS AND METHODS.

Strain	Label ^a	Counts per min in			Mole per cent 6-meA
		Adenine	6-meA	5-meC	
JC12 (<i>dam⁺</i>)	A	614334	9713	—	0.38 ^b
GM81 (<i>dam-3</i>)	A	722891	1164	—	0.04
GM44 (<i>dam⁺</i>)	M	—	1161	922	0.38 ^b
GM45 (<i>dam-3</i>)	M	—	286	1191	0.06
λ CI ₈₅₇ S ₇ (GM44)	M	—	1684	1043	0.22 ^c
λ CI ₈₅₇ S ₇ (GM45)	M	—	262	1148	0.03
fd(F42/GM31)	A	286828	3067	—	0.06
fd(F42/GM48)	A	250687	471	—	0.01

^a A, adenine; M, methionine.

^b The mole per cent 6-meA was calculated on the basis of the adenine content of DNA as 25 mole per cent.

^c The mole per cent of adenine in λ DNA was taken from the literature to be 0.22 (ref. 12).

mined in *dam-3* host cells, since S. HATTMAN (personal communication) has found that such phage DNA appears to lack 6-meA. The data in Table I confirm this observation and show that fd grown in a *dam+* host contains approximately four 6-meA residues per DNA molecule but only 0.1 6-meA residue per molecule if the phage is propagated in a *dam-3* strain. Phage fd propagated in *dam-3* or *dam-3 hsp-1* strains is not restricted in *hsp+* *dam+* hosts (data not shown).

(b) *Phage sensitivity of dam-3 bacteria*

The efficiency of plating of the following phages is the same on *dam+* and *dam-3* strains: T3, T4, T6, T7, T3sam⁻, fd, Φ X174tB, λ , λ red-3, λ gam-210, T4amH39X and T4amE605. Phage λ CI₈₅₇ is efficiently induced at 42° in *dam-3* strains.

(c) *Spontaneous induction in λ lysogens*

The frequency of spontaneous induction of λ in a lysogen of strain W3110, was found to be $1.4 \cdot 10^{-2}$ (Table III). In contrast, the isogenic *dam-3* lysogen produced phage at a frequency of $3.0-3.6 \cdot 10^{-1}$ (Table III). The *dam-3* mutation, therefore, increased the spontaneous induction of λ from lysogens.

TABLE III

SPONTANEOUS INDUCTION OF λ PHAGE FROM *dam-3* AND *dam+* LYSOGENS

Samples were removed from lysogenic strains in the logarithmic phase of growth and assayed for free phage and viable bacteria as described in MATERIALS AND METHODS.

Strain	Viable count/ml	Free phage/ml	Spontaneous frequency
GM28 (λ)	$1.6 \cdot 10^8$	$2.2 \cdot 10^8$	$1.4 \cdot 10^{-2}$
GM33 (λ)	$4.2 \cdot 10^7$	$1.5 \cdot 10^7$	$3.6 \cdot 10^{-1}$
	$2.0 \cdot 10^8$	$6.0 \cdot 10^7$	$3.0 \cdot 10^{-1}$

(d) *Methyl methanesulfonate (MMS) treatment*

The *dam-3* bacteria are more sensitive to UV-irradiation and mitomycin C compared to *dam+* bacteria²⁰. The data in Fig. 1 show that the *dam-3* bacteria are also more sensitive to MMS than *dam+* bacteria. There was no loss in viability of *dam-3* cells during the time of holding in the medium without MMS.

(e) *Spontaneous and induced mutagenesis in dam-3 strains*

The *dam-3* mutation increases the spontaneous mutation frequency by 7-46-fold, depending on the marker selected, compared to *dam+* strains²⁰. None of the markers tested previously, however, are suppressible by *amber* or *ochre* suppressors, and therefore, the frequency of reversion of *lacZ608*, an *amber* mutation, and *his-4*, an *ochre* mutation, was determined. The data in Table IV show that there is a 129-fold increase in Lac⁺ reversion and a 15-fold increase in His⁺ reversion in *dam-3* strains compared to the *dam+* control.

Since *dam-3* increases the spontaneous reversion frequency, it was of interest to determine if the UV-irradiation induced mutation frequency was also increased. The rate of Lac⁺ reversion in strains GM67 (*dam+*) and GM68 (*dam-3*) after exposure to varying doses of ultra-violet irradiation, however, was the same (data not shown).

(f) *Survival of dam uvr mutants after UV-irradiation*

The increased sensitivity after UV-irradiation of *uvr rec* strains compared to

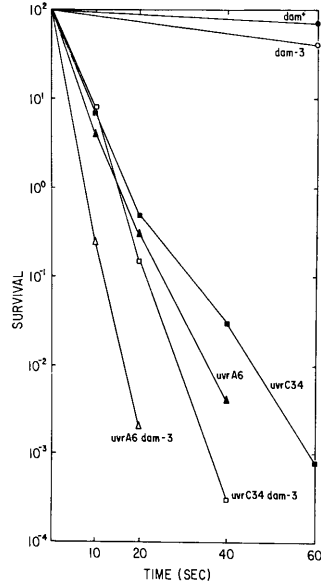
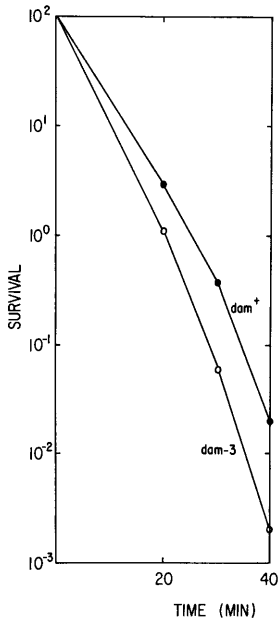


Fig. 1. Survival of *dam-3* and *dam+* bacteria to MMS. Log phase cultures were diluted in minimal medium containing 0.15 M NaCl and 0.05 M MMS. At various times samples were removed and survival determined as described in MATERIALS AND METHODS.

Fig. 2. Survival after UV-irradiation of AB1884 (*uvrC34*), AB2500 (*uvrA6*), GM55 (*dam-3 uvrA6*) and GM73 (*dam-3 uvrC34*). Stationary-phase cultures were diluted in minimal medium, irradiated at a distance of 100 cm from the light source and plated on complete medium to determine survival.

TABLE IV

REVERSION FREQUENCY OF *amber* AND *ochre* MUTATIONS IN *dam+* AND *dam-3* STRAINS

An equal number of viable cells from stationary-phase cultures of GM69 and GM85 were diluted and plated on minimal medium to select for Lac⁺ or His⁺ revertants. The plates were scored after 4 days incubation at 37°.

Strain	Reversion frequency of	
	<i>lacZ608</i>	<i>his-4</i>
GM69 (<i>dam+</i>)	4.8 · 10 ⁻⁸	2.7 · 10 ⁻⁸
GM85 (<i>dam-3</i>)	6.2 · 10 ⁻⁶	4.1 · 10 ⁻⁷
<i>dam-3/dam+</i>	129	15

uvr rec+ and *uvr+ rec* parental strains, has been interpreted to mean that the *uvr* and *rec* genes are involved in different steps of dark repair of UV-induced lesions in DNA¹³. Since bacteria containing *dam-3* in combination with *uvrA6* and *uvrC34* are viable, it was of interest to determine the survival of these double mutants after UV-irradiation. The data in Fig. 2 show that the *dam-3 uvrA6* and *dam-3 uvrC34* mutants are more sensitive to UV-irradiation than the *dam+* *uvr* parental strains. The result suggests that the UV-sensitivity associated with *dam-3* is independent of that for *uvrA* and *uvrC* gene function.

(g) Post-replication repair in *dam-3* bacteria

In addition to excision repair, *E. coli* can repair UV-damaged DNA by a mecha-

TABLE V

MOLECULAR WEIGHT OF DNA IN *dam* AND *dam-3* STRAINS AFTER UV-IRRADIATION

Post replication repair was determined as described by RUPP AND HOWARD-FLANDERS²⁵. The alkaline sucrose gradients were centrifuged in an SW39 rotor at 30000 rev./min for 75 min at 20°.

Source of DNA	Molecular weight $\cdot 10^{-6a}$	
	AB2500 (<i>dam</i> ⁺ <i>uvrA6</i>)	GM55 (<i>dam-3</i> <i>uvrA6</i>)
120 min labeling, no UV-irradiation	195	195
Labeled 10 min after UV-irradiation	16	16
Labeled 10 min after UV-irradiation plus 70 min in non-radioactive medium	150	150

^a The molecular weight of DNA was calculated using the single strand molecular weight of λ DNA, 16×10^6 , as reference.

nism involving DNA replication and subsequent recombination²⁵. This mechanism of post-replication repair occurs as efficiently in *dam-3* bacteria as in *dam*⁺ bacteria (Table V). This result was not unexpected since *dam-3* has no effect on recombination proficiency²⁰.

(h) Inviability of dam-3 lex-1 cells

The *lex* gene maps close to *uvrA* and mutations in this gene render the cell sensitive to UV-irradiation, reduces the spontaneous induction of λ from lysogens, and abolishes UV-induced mutagenesis²³. We attempted to construct a *dam-3 lex-1* strain to determine if *lex-1* would abolish the phenotypic traits associated with *dam-3*.

When Hfr GM42 (*dam-3 xyl*⁺) was mated with AB2500 (*xyl-5 uvrA6*), 60% (15/25) of the Xyl⁺ recombinants were *dam-3 uvrA6*. When Hfr GM42 was mated with DM803 (*xyl-5,7 lex-1*), however the number of Xyl⁺ recombinants was only 10–20% of that in the GM42 \times AB2500 cross. Furthermore, none (0/40) of these recombinants had the *dam-3 lex-1* genotype, but all were either *dam-3 lex*⁺ or *dam*⁺ *lex-1*. This result suggests that *dam lex* bacteria are inviable. Further evidence for this was obtained from transductional crosses. A Plvir lysate was prepared from DM803 (*lex-1*) and used to transduce GM69 (*dam*⁺ *dnaB43*) to *DnaB*⁺. 78% (29/37) of these transductants were *lex-1*. The same lysate was mixed with GM75 (*dam-3 dnaB43*) and selection made for *DnaB*⁺. The transduction frequency was ten-fold lower and no (0/20) *dnaB43*⁺ *lex-1* bacteria were recovered. These results are interesting since bacteria containing *dam-3* in combination with *recA* or *recB,C* or *polA* are also inviable²⁰.

(i) Susceptibility of methyladenine-deficient DNA to alkali denaturation

The DNA of *dam-3* bacteria contains single strand breaks as judged by sedimentation in sucrose gradients after alkali denaturation²⁰. The DNA of phage λ , propagated on a *dam-3* host, does not appear to contain single-strand breaks, even though such DNA is undermethylated with respect to adenine (Fig. 3). Furthermore, this experiment shows that methylation-deficient DNA and fully methylated DNA are equally susceptible to alkali denaturation.

(j) Molecular weight of DNA in dam-3 lig-7 bacteria

Bacteria bearing the *lig-7* mutation produce a temperature-sensitive DNA ligase^{9,14,24} and the molecular weight of DNA in *dam-3 lig-7* bacteria cultured at 42° is lower than that of *dam*⁺ *lig-7* strains²⁰. Fig. 4 shows the kinetics of DNA degradation in

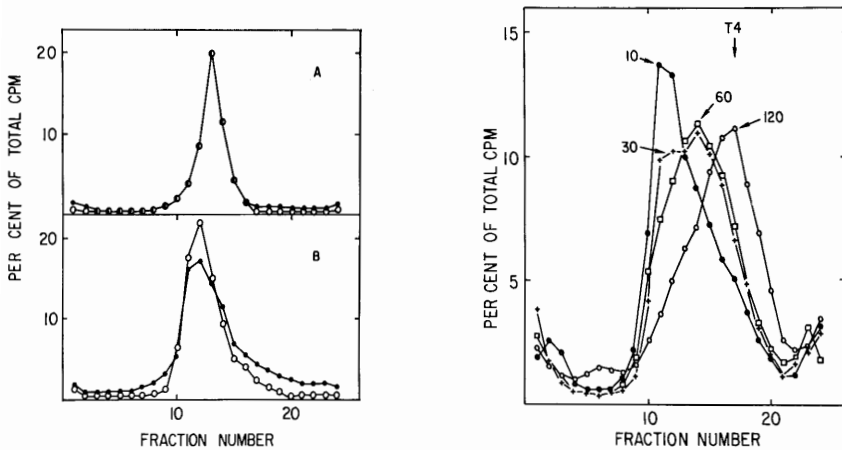


Fig. 3. Sedimentation in neutral sucrose gradients of fully methylated ^3H - λ DNA (\bullet) and methyladenine-deficient ^{14}C - λ DNA (\circ). A, Untreated; B, DNA was denatured in 0.3 M NaOH for 10 min at room temperature. The gradients were centrifuged in an SW39 rotor at 30000 rev./min for 240 min at 20° . ^3H counts/min = 2523; ^{14}C counts/min = 281. Sedimentation in this and all subsequent figures is from right to left.

Fig. 4. Sedimentation in alkaline sucrose of DNA from GM66 (*dam-3 lig-7*). Strain GM66 was grown in K medium with [^{14}C]thymidine at 30° for 120 min. The culture was harvested, washed and resuspended in non-radioactive K medium at 42° . At various times samples were removed, the bacteria lysed and portions of the lysate, together with ^3H -T4 DNA, sedimented in alkaline sucrose gradients. The gradients were centrifuged in an SW39 rotor at 30000 rev./min for 75 min at 20° . ^{14}C counts/min in 0, 30, 60 and 120 min samples were 16705; 19082; 14564 and 15215 respectively.

GM66 (*dam-3 lig-7*) cultured at 42° . The molecular weight of DNA at 10, 60 and 120 min after raising the temperature is 180 , 99 and $55 \cdot 10^6$ respectively. In contrast no such decrease in molecular weight was observed in *dam+ lig-7* strains (data not shown.)

The limit molecular weight of DNA in *dam-3 lig-7* bacteria appears to be $55 \cdot 10^6$ since further incubation of the strain at 42° does not reduce the molecular weight. This DNA product, however, might not be a true limit but may represent an equilibrium between (1) DNA degradation and DNA synthesis or (2) DNA degradation and DNA post-replication repair or (3) a combination of the above. To test this possibility, a *dnaB* mutation was introduced into *dam-3 lig-7* and *dam+ lig-7* strains. The *dnaB* mutation causes an immediate cessation of DNA replication if the strain is cultured at 42° (ref. 3) and since no daughter DNA strands can be synthesized, post-replication repair should be abolished. The data in Fig. 5 show that the molecular weight of DNA in *dam-3 lig-7* bacteria in the absence of DNA replication reaches a limit size 60 min after raising the temperature, co-sediments with single-stranded T4 DNA (molecular weight $55 \cdot 10^6$) and is not reduced by further incubation. In the control *dam+ lig-7* culture the DNA molecular weight distributions are more heterogeneous and the bulk of the DNA sediments faster than single-stranded T4 DNA (Fig. 6). In both strains an appreciable amount of the DNA is degraded to acid-soluble material but the rate of degradation is the same (data not shown). This degradation is probably due to the *recB,C* nuclease since BUTTIN AND WRIGHT⁶ have shown that DNA degradation in *dnaB* strains can be reduced by the introduction of a *recB,C* mutation. It is clear from the above data, however, that the $55 \cdot 10^6$ DNA product cannot represent an equilibrium involving DNA synthesis, degradation or post-replication repair.

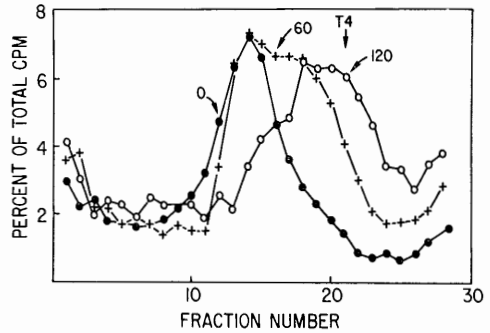
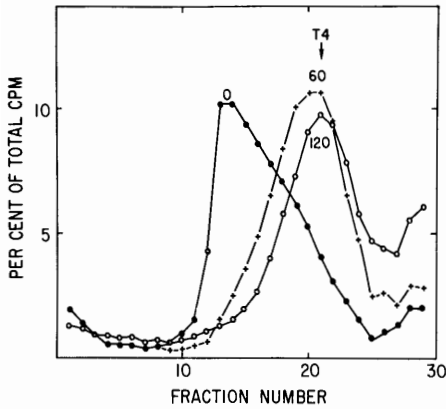


Fig. 5. Sedimentation in alkaline sucrose of DNA from strain GM79 (*dam-3 lig-7 dnaB43*). The experimental procedure was the same as that described for Fig. 4. ¹⁴C counts/min in 0, 60 and 120 min samples were 8734; 8032 and 5363 respectively.

Fig. 6. Sedimentation in alkaline sucrose of DNA from strain GM83 (*dam+ lig-7 dnaB43*). The experimental procedure was the same as that described for Fig. 4. ¹⁴C counts/min in 0, 60 and 120 min samples was 13558; 12467 and 7932 respectively.

(k) DNA degradation in *dam-3 recA200* bacteria

Bacteria mutant in the *dam* gene degrade DNA to acid-soluble material at the same rate as *dam+* bacteria (Fig. 7). Similarly no difference in the rate of degradation has been observed in *dam+* or *dam-3* double mutants also containing *recBtsI*, *recCtsI*, *polA12* or *lig-7* (data not shown). In contrast, *dam-3 recA200* bacteria degrade DNA to acid-soluble material at a faster rate than *dam+ recA200* cells (Fig. 7).

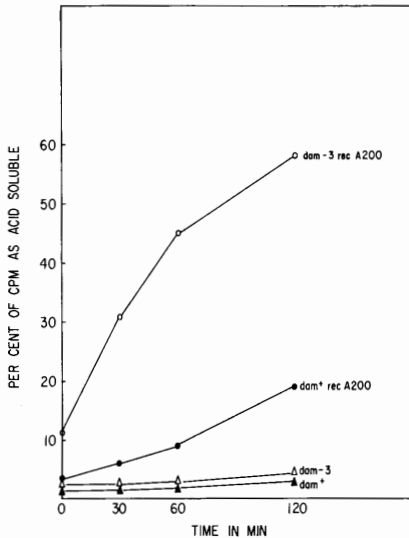


Fig. 7. DNA degradation in *dam-3* and *dam+* strains. The bacteria were grown in K medium with [³H]- or [¹⁴C]thymidine at 30° for 120 min. The cells were harvested, washed and resuspended in K medium at 42°. At the times indicated, samples were removed and assayed for acid-soluble and acid-insoluble radioactivity.

The *recA200* mutation is a conditional mutation recently described by LLOYD *et al.*¹⁵ which is Rec⁻ at temperatures above 39°, but retains viability. The *dam-3 recA200* strain is not viable at 42° possibly because of the observed DNA breakdown. In this context, MONK AND KINROSS²² and LLOYD *et al.*¹⁵ have demonstrated that *polA12 recA200* strains are inviable at 42° and degrade a significant amount of DNA to acid-soluble material at this temperature.

DISCUSSION

The *dam-3* mutant differs from *dam*⁺ bacteria in the following respects: (i) decreased DNA adenine methylase activity *in vitro* and *in vivo*, (ii) irregular morphology of *dam-3* cells, (iii) increased sensitivity to UV-irradiation, mitomycin C and MMS, (iv) increased free phage in *dam-3* lysogenic cultures, (v) increased generalized spontaneous mutability, (vi) inviability of strains bearing *dam-3* and *recA*, *recB,C*, *polA* or *lex-I*, (vii) decreased ability to restrict foreign DNA, (viii) increased number of single-strand breaks in DNA which are amplified in the absence of DNA ligase or DNA polymerase I and (ix) increased DNA breakdown in *dam recA* bacteria. These facts would lead to the conclusion that a function of 6-meA is to protect DNA from nucleolytic degradation²⁰.

The primary defect in *dam-3* bacteria is inability to form normal amounts of DNA adenine methylase, which results in adenine undermethylation of DNA (Table II). It should be noted, however, that the *dam-3* mutation is "leaky" and strains carrying this mutation still contain 15% of the wild type level of 6-meA in DNA. That phage λ and fd DNA are undermethylated in *dam-3* hosts, indicates that adenine methylation of phage DNA is a host-controlled function. The finding that fd DNA contains only 0.1 6-meA residues per molecule, if the phage is propagated in a *dam-3* strain, would mean that this DNA is a substrate for the *dam* DNA adenine methylase but not the *hsp* DNA adenine methylase which imparts K12 host specificity. This would explain why fd is not restricted by K12 bacteria¹ since there are no base sequences in fd DNA recognized by endonuclease III of *E. coli* K12 and the DNA would be resistant to degradation. fd propagated on a *dam-3* host is not restricted if plated on a *dam*⁺ indicator. Possible explanations for this are that (i) as soon as the undermethylated fd DNA enters the cell, it is methylated by the *dam* DNA adenine methylase and hence escapes restriction by the hypothetical endonuclease acting on 6-meA-deficient DNA. This may mean that the methylase and endonuclease have different affinities for substrate DNA molecules, (ii) fd DNA contains no base sequences recognized by the endonuclease or (iii) the endonuclease introduces breaks in fd DNA but such breaks are repaired by repair enzymes.

The *dam-3* mutant shows increased prophage induction, mutability and decreased septation compared to *dam*⁺ cells. Such phenotypic traits can also be induced in *dam*⁺ bacteria irradiated with ultra-violet light providing the *recA* and *lex* gene products are present²⁸. It is possible, therefore, that these traits in *dam-3* bacteria are consequences secondary to DNA damage. A test of this hypothesis would be to examine a *dam-3 lex-I* mutant in which these traits should be abolished. Unfortunately, the results in section (h) show that such strains are inviable. Studies with the *dam-3 recA200* mutant may partially answer this question.

The basis for increased sensitivity of *dam-3* mutants to UV-irradiation, mitomycin C, MMS and decreased restriction of foreign DNA, is not known. In exponentially

growing *dam-3* bacteria, however, there are single strand breaks in DNA which are constantly being repaired by DNA repair enzymes²⁰. If a *dam-3* strain is subjected to UV-irradiation, mitomycin C, MMS or unmodified λ DNA, these enzymes may become saturated and consequently the process of repair and restriction would not be as efficient as in a *dam+* strain. Thus the sensitivity of *dam-3* bacteria to these deleterious agents would be a secondary consequence due to saturation of repair enzymes rather than a primary consequence of adenine undermethylation. The involvement of repair enzymes in restriction has been demonstrated by SIMMON AND LEDERBERG²⁶.

The data presented in this and the companion paper²⁰ show that a function of 6-meA is to protect DNA from an endonuclease. The identity of this hypothetical endonuclease is not known but endonuclease III, the K12 restriction enzyme, has been ruled out²⁰. The hypothetical endonuclease is not *endA* or the *uvrA,B* endonuclease. Bacteria mutant for *dam-3* and *endA* contain more breaks in DNA than *dam+ endA* strains, showing that the hypothetical endonuclease is active in these cells. Similarly, *uvrA6 dam-3* bacteria contain more breaks in DNA than *dam+ uvrA6* cells. Currently, we are attempting to detect an endonucleolytic activity in crude extracts of *E. coli* able to degrade methyladenine deficient DNA.

The data in section (k) show that single-strand breaks in DNA occur in *dam-3* bacteria in the absence of DNA ligase. The degradation product appears to have a single-strand molecular weight of $55 \cdot 10^6$ and is not an equilibrium between DNA degradation and DNA synthesis or between DNA synthesis and a post-replication mechanism of DNA repair. The high molecular weight of the product is surprising since if each normally methylated adenine can be a site for breakage, the limit single strand molecular weight of DNA in *dam-3 lig* strains should be about $1 \cdot 10^5$. This discrepancy in molecular weight might be due to the leakiness of the *dam-3* mutation or perhaps to residual DNA repair, or to some feature of chromosomal structure.

The increased DNA degradation in *dam-3 recA200* strains compared to *dam+ recA200* control was surprising. Such increased DNA degradation has also been found in *polA recA* bacteria^{15,22}. The basis for this degradation is not known. Perhaps the single-strand breaks in DNA of *dam-3* bacteria, in the absence of the *recA* gene product, become sensitive to nucleolytic degradation. This may mean that the *recA* gene product functions to stabilize breaks in DNA and prevent degradation by extraneous nucleases.

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REFERENCES

- 1 ARBER, W., Host specificity of DNA produced by *Escherichia coli* ϕ 9. Host controlled modification of bacteriophage fd, *J. Mol. Biol.*, 20 (1966) 483.
- 2 ARBER, W., Host-controlled restriction and modification of bacteriophage, *18th Symp. Soc. Gen. Microbiol.*, Cambridge University Press, London, 1968, pp. 295-314.
- 3 BONHOEFFER, F., DNA transfer and DNA synthesis during bacterial conjugation, *Z. Vererbungsleh.*, 98 (1966) 141.
- 4 BOVRE, K., W. SZYBALSKI, Patterns of convergent and overlapping transcription within the b2 region of coliphage λ , *Virology*, 38 (1969) 614.

- 5 BOYER, H. W., DNA restriction and modification mechanisms in bacteria, *Ann. Rev. Microbiol.*, 25 (1971) 153.
- 6 BUTTIN, G., AND M. R. WRIGHT, Enzymatic DNA degradation in *E. coli*. Its relationship to synthetic processes at the chromosomal level, *Cold Spring Harbor Symp. Quant. Biol.*, 33 (1968) 259.
- 7 CLARK, A. J., M. CHAMBERLIN, R. P. BOYCE AND P. HOWARD-FLANDERS, Abnormal metabolic response to ultraviolet light of a recombination-deficient mutant of *Escherichia coli* K12, *J. Mol. Biol.*, 19 (1966) 442.
- 8 DAVIS, B. D., AND E. S. MINGIOLI, Mutants of *Escherichia coli* requiring methionine or vitamin B12, *J. Bacteriol.*, 60 (1951) 17.
- 9 GOTTESMAN, M. M., M. L. HICKS AND M. GELLERT, Genetics and function of DNA ligase in *Escherichia coli*, *J. Mol. Biol.*, 77 (1973) 531.
- 10 GOUGH, M., AND S. LEDERBERG, Methylated bases in the host-modified deoxyribonucleic acid of *Escherichia coli* and bacteriophage λ , *J. Bacteriol.*, 91 (1966) 1460.
- 11 HATTMAN, S., Plasmid-controlled variation in the content of methylated bases in bacteriophage lambda deoxyribonucleic acid, *J. Virol.*, 10 (1972) 356.
- 12 HATTMAN, S., S. SCHLAGMAN AND L. COUSENS, Isolation of a mutant of *Escherichia coli* defective in cytosine-specific deoxyribonucleic acid methylase activity and in partial protection of bacteriophage λ against restriction by cells containing the N-3 drug resistance factor, *J. Bacteriol.*, 115 (1973) 1103.
- 13 HOWARD-FLANDERS, P., DNA repair, *Ann. Rev. Biochem.*, 37 (1968) 175.
- 14 KONRAD, E. B., P. MODRICH AND I. R. LEHMAN, Genetic and enzymatic characterization of a conditional lethal mutant of *Escherichia coli* K12 with a temperature-sensitive DNA ligase, *J. Mol. Biol.*, 77 (1973) 519.
- 15 LLOYD, R. G., B. LOW, G. N. GODSON AND E. A. BIRGE, The isolation and characterization of a mutant of *Escherichia coli* K-12 with a temperature sensitive RecA⁻ phenotype, *J. Bacteriol.*, 120 (1974) 407.
- 16 MAMELAK, L., AND H. W. BOYER, Genetic control of the secondary modification of DNA in *Escherichia coli*, *J. Bacteriol.*, 104 (1970) 57.
- 17 MARINUS, M. G., Location of DNA methylation genes on the *Escherichia coli* K-12 genetic map, *Mol. Gen. Genet.*, 127 (1973) 47.
- 18 MARINUS, M. G., AND E. A. ADELBERG, Vegetative replication and transfer replication of deoxyribonucleic acid in temperature-sensitive mutants of *Escherichia coli* K-12, *J. Bacteriol.*, 104 (1970) 1266.
- 19 MARINUS, M. G., AND N. R. MORRIS, Isolation of DNA methylase mutants from *Escherichia coli* K-12, *J. Bacteriol.*, 114 (1973) 1143.
- 20 MARINUS, M. G., AND N. R. MORRIS, Biological function for 6-methyladenine residues in the DNA of *Escherichia coli* K-12, *J. Mol. Biol.*, 85 (1974) 309.
- 21 MESSELSOHN, M., R. YUAN AND J. HAYWARD, Restriction and modification of DNA, *Ann. Rev. Biochem.*, 41 (1972) 447.
- 22 MONK, M., AND J. KINROSS, Conditional lethality of *recA* and *recB* derivatives of a strain of *Escherichia coli* K-12 with a temperature sensitive deoxyribonucleic acid polymerase I, *J. Bacteriol.*, 109 (1972) 971.
- 23 MOUNT, D. W., K. B. LOW AND S. J. EDMISTON, Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations, *J. Bacteriol.*, 112 (1972) 886.
- 24 PAULING, C., AND L. HAMM, Properties of a temperature-sensitive radiation-sensitive mutant of *Escherichia coli*, *Proc. Natl. Acad. Sci. (U.S.)*, 60 (1968) 1495.
- 25 RUPP, W. D., AND P. HOWARD-FLANDERS, Discontinuities in the DNA synthesized in an excision-defective strain of *Escherichia coli* following ultraviolet irradiation, *J. Mol. Biol.*, 31 (1968) 291.
- 26 SIMMON, V. F., AND S. LEDERBERG, Degradation of bacteriophage lambda deoxyribonucleic acid after restriction by *Escherichia coli* K-12, *J. Bacteriol.*, 112 (1972) 161.
- 27 TAYLOR, A. L., AND C. D. TROTTER, Linkage map of *Escherichia coli* strain K-12, *Bacteriol. Rev.*, 36 (1972) 504.
- 28 WITKIN, E., Thermal enhancement of ultraviolet mutability in a *tif-1 uvrA* derivative of *Escherichia coli* B/r: Evidence that ultraviolet mutagenesis depends upon an inducible function, *Proc. Natl. Acad. Sci. (U.S.)*, 71 (1974) 1930.
- 29 VAPNEK, D., AND W. D. RUPP, Asymmetric segregation of the complementary sex-factor DNA strands during conjugation in *Escherichia coli*, *J. Mol. Biol.*, 53 (1970) 287.