

Hyper-recombination in *dam* Mutants of *Escherichia coli* K-12

M.G. Marinus*¹ and E. Bruce Konrad

¹ Dept. of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01605
 and Dept. of Biochemical Pharmacology, N.I.A.M.D.D., National Institutes of Health, Bethesda, MD 20014

Summary. F-prime heterogenotes of *dam-3* bacteria segregate F-prime homogenotes at a frequency 30–200 times higher than the isogenic *dam*⁻ strain. A hyper-recombination mutant which shows increased recombination between chromosomal duplications was characterized as a *dam* mutant. The *dam-3* allele causes a reduction in linkage of proximal unselected markers in transconjugants and increases the recombination frequency between a pair of closely linked markers. It is concluded that *dam* mutations confer a hyper-recombination phenotype to the cell.

1. Introduction

Strains of *Escherichia coli* K-12 mutant in the *dam* gene have 1. reduced amounts of a DNA adenine methylase which results in adenine undermethylation of DNA (Marinus and Morris, 1973); 2. contain excess single strand breaks in DNA and 3. show a variety of pleiotropic effects which include increased sensitivity to ultra-violet light, a high frequency of spontaneous mutagenesis and a high frequency of spontaneous induction of lysogenic phages (Marinus and Morris, 1974; 1975). Data are presented in this communication which suggest that *dam* mutants also exhibit a hyper-recombination (hyper-rec) phenotype.

2. Materials and Methods

a) Bacterial Strains

The *E. coli* K-12 strains used in this study are described in Table 1. The points of origin of the various Hfr strains and some pertinent genetic markers are shown in Figure 1.

* To whom reprint requests should be sent

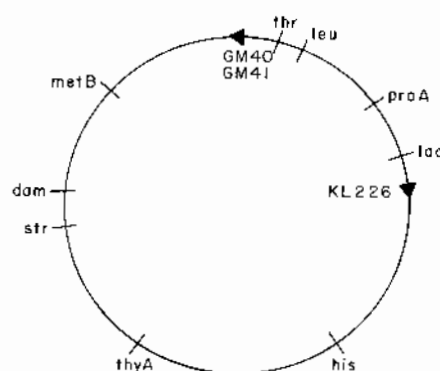


Fig. 1. Genetic map of *E. coli* K-12 (after Bachmann et al., 1976) showing pertinent genetic markers on the outside of the circle and the points of origin of various Hfr strains on the inside of the circle

b) Isolation Characterization of Putative Spontaneous Deletions in F141

Stationary phase cultures of strain F141/JC1553 were diluted and portions plated on McConkey medium supplemented with maltose as sole carbon source and 100 µg streptomycin per ml. Twenty nine *Mal*⁻ *Str*^r clones were obtained and after purification cross-streaked against recipients to select for the ability to transfer *argG*, *aroE*, *cysG*, *trpS*, *aroB*, *axd* and *mal*. Figure 2 shows the results with three such F141 derivatives: F141-19, F141-9, and F141-17. The markers enclosed by the rectangles are not transferred by the particular F-prime. It is presumed that the genetic material enclosed by the rectangles has been deleted although no physical measurements have been made to verify this.

c) Media

The minimal medium was that of Davis and Mingioli (1951). Complete medium was either Difco brain heart infusion broth (20g/L) or Luria broth (Luria and Burrous, 1957). Antibiotic medium no. 2 and McConkey Agar Base were obtained from Difco. When required, the media were solidified with 1.6% Difco agar.

Table 1. List of *E. coli* strains used in this study

Strain	Sex	Genotype ^a	Derivation
AB1874	F'	<i>Flac</i> ⁺ / <i>lac-19</i>	CGSC ^b
AB2605	F'	<i>Fgal</i> ⁺ / <i>galT12</i>	CGSC
F42/GM44	F'	<i>Flac</i> ⁺ / <i>thr-1 leu-6 proA2 metB1 lacY1 galK2 ara-14 tsx-33 thi-1 thyA12 deoB16 supE44</i>	AB1874 × GM44
F42/GM45	F'	As F42/GM44 but <i>dam-3</i>	AB1874 × GM45
F8/GM67	F'	<i>F-gal</i> ⁺ / <i>lacZ4 thi-1 galK2 tsx-6</i>	AB2605 × GM67
F8/GM68	F'	As F8/GM67 but <i>dam-3</i>	AB2605 × GM68
F42/GM90	F'	<i>trpS10330, lac</i> ⁻ <i>str</i> ^R	This laboratory
F141/JC1553	F'	<i>F-argG</i> ⁺ <i>-asd</i> ⁺ / <i>argG6 metB1 his-1 leu-6 recA1 mtl-2 xyl-7 malA1 gal-6 lacY1 str-104 supE44</i>	CGSC
GM40	Hfr	<i>thi-1 rel-1</i>	AB4506
GM41	Hfr	As GM40 but <i>dam-3</i>	AB4506
KL226	Hfr	<i>rel-1 tonA2</i>	CGSC
KS391	Hfr	<i>lacMS286 φ 80dII lacBK1 thi</i> ⁻	E.B. Konrad
RS5033	Hfr	As KS391 but <i>dam-4</i>	E.B. Konrad
AB266	F ⁻	<i>thr-1 leu-6 proA2 lacY1 galK2 ara-14 str-20 xyl-5 mtl-1 thi-1</i>	CGSC
GM112	F ⁻	<i>thr-1 leu-6 proA2 his-4 metB1 lacY1 galK2 ara-14 tsx-33 thi-1 thyA12 deoB16 supE44 mtl-1? str-31</i>	This laboratory
GM113	F ⁻	As GM112 but <i>dam-3</i>	This laboratory
KL251	F ⁻	<i>thi</i> ⁻ <i>leu</i> ⁻ <i>metE</i> ⁻ <i>proC</i> ⁻ <i>purE</i> ⁻ <i>trp</i> ⁻ <i>recA</i> ⁻ <i>str</i> ^R <i>ara</i> ⁻ <i>xyl</i> ⁻ <i>az</i> ^R <i>lac</i> ^Z <i>ton</i> ^R <i>tsx</i> ^R	K.B. Low

^a Unless stated otherwise, all strains are λ^- , λ^S

^b *E. coli* Genetic Stock Center, Department of Human Genetics, Yale University School of Medicine, New Haven, CT 06510 (U.S.A.)

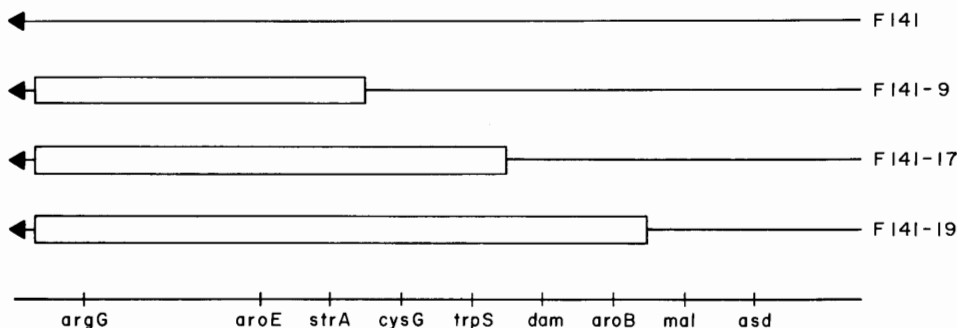


Fig. 2. Putative deletion derivatives of F141. Markers within the rectangle are not transferred. The genetic map distances are not drawn to scale

d) Genetic Procedures

Methods for the preparation of crude extract and scoring of *dam* recombinants have been described previously (Marinus, 1973). The conjugation experiments to be described were carried out as follows: Donors and recipients in the logarithmic phase of growth growing in brain heart broth were mixed to give $1-2 \times 10^7$ donors/ml and $2-4 \times 10^8$ recipients per ml and incubated without shaking for 60 min in brain heart broth plus 40 μ g thymine/ml. The mating mixtures were blended for 60 s and phage T6 added at a multiplicity of 100 phage/donor cell. After 30 min the cells were diluted one thousandfold into brain heart broth containing 40 μ g thymine/ml and grown with aeration for 3 h before plating on the appropriate minimal medium 40 μ g thymine/ml. Control experiments showed that there were no detectable recombinants formed during incubation with a T6 treated male.

Results

a) Map Location of the *dam-3* Mutation

In a previous study (Marinus, 1973) the gene order *cysG*-(*trpS*, *dam*)-*aroB* was inferred on the basis of two factor crosses by phage P1 transduction. The data from these crosses were necessarily crude since only ten transductants in each recombinant class could be scored. To determine the gene order precisely, a series of putative deletion mutations in the F-prime plasmid F141 were isolated as described in Materials and Methods. Some of these putative deletions are shown in

Table 2. Incorporation of methyl groups into wild type and mutant DNA by a crude extract of *E. coli* K-12

Source of DNA	Counts/min/10 ml of culture
GM100	22,401
F141/GM100	248
F141-9/GM100	236
F141-17/GM100	217
G141-19/GM100	23,620

DNA was isolated from bacterial cultures and assayed for ability to accept methyl groups into DNA as described previously (Marinus and Morris, 1973). The results are the average of three separate determinations.

Figure 2. The F141 derivative plasmids were transferred into strain GM100 (*dam-3 mal-354*) and DNA extracted from the purified Mal⁺ plasmid transconjugants and tested for ability to accept methyl group transfer into DNA in vitro. Table 2 shows that the DNA extracted from F141-9/GM100 and F141-17/GM100 heterogenotes is fully methylated whereas the DNA from F141-19/GM100 is undermethylated. Correlating these data with that in Figure 2, it is concluded that the gene order is *cysG-trp-dam-aroB*. This gene order has recently been confirmed by the use of three factor crosses (Kushner, personal communication). Attempts to cure *dam-3* bacteria of the F141 derivatives with acridine orange were unsuccessful.

b) Homogenote Formation from F-prime Heterogenotes

The appearance of homogenotes from F-prime heterogenotes has been used as a measure of recombination. This conversion normally occurs at low frequency (Jacob and Wollman, 1961) and appears to require recombination since it is abolished in *recA*⁻ bacteria (Low, 1972). It was reasoned that a mutation causing hyper-recombination should lead to an increased frequency of homogenote segregation from heterogenotes.

Various F-prime heterogenotes of *dam*⁺ and *dam-3* strains were constructed and in every case, homogenote formation was at least thirty fold higher in the mutant than the otherwise isogenic wild type strain. Two examples are given in Table 1. Homogenote formation was unaffected by the temperature of incubation (30 C, 37 C, 42 C), or the presence or absence of suppressor mutations.

The Lac⁻ segregants from a F-*lac*⁺/*lac*⁻ *dam-3* strain were shown to be F-*lac*⁻/*lac*⁻ homogenotes by the following criteria. One hundred Lac⁻ segregants from strain F42/GM45 Pro⁺ (F-*lac*⁺/*lacY1 dam-3 proA*⁻ *str*^S) were isolated, purified and cross-streaked (Marinus, 1974) against AB266 (F *proA2 str*^R). All

Table 3. Frequency of homogenote formation from *dam*⁺ and *dam-3* F-prime heterogenotes

Strain	Genotype	Selection	Frequency (%)
F42/GM44	<i>dam</i> ⁺	Lac ⁻	<0.1
F42/GM45	<i>dam-3</i>	Lac	10-20
F8/GM67	<i>dam</i> ⁺	Gal	<0.1
F8/GM68	<i>dam-3</i>	Gal ⁻	3-6

Stationary phase cultures were diluted and plated on either McConekey agar or Difco Antibiotic Medium no. 2 containing tetrazolium. The plates were incubated for 18 h at 37 C before scoring. Bi-sectioned colonies were scored as both + and -. F42 is the F-*lac* episome; F8 is F-*gal*.

the Lac⁻ segregants sired Pro⁻ Str^R recombinants, showing that they were male bacteria. To discriminate between F-prime and Hfr males, advantage was taken of the observation that Hfr × F⁻ *recA*⁻ crosses yield few transconjugants whereas F-prime × F⁻ *recA*⁻ crosses yield plasmid transconjugants at the normal frequency (Clark and Margulies, 1965). All the Lac⁻ *dam-3* segregants produced Lac⁺ recombinants when cross-streaked against KL251 (*lacZ36 recA1*) to the same extent as the control strain, AB1874 (F-*lac*⁺/*lacY1 dam*⁺). The yield of Lac⁺ recombinants with Hfr KL225 in the cross with KL251 was sharply reduced. It was concluded that the genotype of the *dam-3* Lac⁻ segregants was F-*lacY1/lacY1*. Using similar methods it has been shown that Lac⁻ segregants from F42/GM68 (*dam-3 lacZ4*) are homogenotes of type F-*lacZ4/lacZ4*. These results suggest that the *dam-3* mutation either increases recombination between plasmid and host chromosome or that it promotes aberrant segregation of plasmids. It was not possible to test the effect of a *recA*⁻ mutation on homogenote formation since a cell containing both *dam*⁻ and *recA*⁻ mutations is inviable (Marinus and Morris, 1974).

c) DNA Adenine Undermethylation in Strain RS5033

If mutations in the *dam* gene cause a hyper-rec phenotype, then conversely, some hyper-rec strains should be *dam* mutants. Such hyper-rec strains have recently been isolated by Konrad (1976). One of these, RS5033, was shown to be a *dam* mutant by the following experiments. 1. The specific activity of DNA adenine methylase in extracts of KS391 (the wild type) and RS5033 (*dam-4*) is 51.3 and 2.2 pmoles methyl groups incorporated/hr/mg protein. 2. The mole per cent 6-methyladenine per 100 adenines in DNA of KS391 and RS5033 is 2.2 and 0.32 respectively. 3. Since the *dam* gene is closely linked to *trpS* (Marinus, 1973), a Plvir lysate of RS5033 was prepared and used to transduce strain F42/GM90 (F-*lac*⁺/*lac*⁻

trpS10330) to *Trp*⁺. Eighty per cent (8/10) of the transductants had adenine undermethylated DNA and showed concomitant segregation of Lac⁻ clones at high frequency. The remaining transductants did not segregate Lac⁻ clones at high frequency and had normal amounts of 6-methyladenine in DNA. It is concluded that RS5033 is a *dam* mutant and that the *dam-4* mutation and the hyper-rec phenotype co-segregate.

d) Chromosome Mobilization by F42 (*F-lac*)

Chromosome mobilization by F-prime plasmids is a *recA* dependent process. (Low, 1972). The frequency of mobilization of the chromosomal gene, *proA* by F42 from a *dam*⁺ donor to a *dam*⁺ or *dam-3* recipient varies from 1.5–6.4 Pro⁺ transconjugants per 100 donors. The frequency of transfer of *proA* by F42 from a *dam-3* donor to *dam*⁺ or *dam-3* recipients varies from 2.0–5.5 Pro⁺ transconjugants per 100 donors. The *dam-3* mutation, therefore, does not affect the frequency of F-prime chromosome mobilization.

e) Influence of the *dam-3* Marker on the Genetic Constitution of Transconjugants

Since the *dam-3* mutation confers a hyper-rec phenotype to the cell, it was of interest to determine if it would have any influence on linkage frequencies of unselected markers in transconjugants.

A series of matings were carried out using *dam*⁺ (GM40) and *dam-3* (GM41) derivatives of Hfr Hayes and strains GM112 (F⁻ *dam*⁺) and GM113 (F⁻ *dam-3*). The latter two strains are isogenic except for the *dam* mutation. After mating the strains in various combinations for 60 min, phage T6 was added to destroy the males and the transconjugants were incubated for 3 h to allow expression and segregation of markers. The cells were then plated and selection made for Pro⁺ recombinants and after purification the transconjugants were scored for the presence of the unselected markers *thr*, *ara* and *leu*. The frequency of Pro⁺ transconjugants per 100 donors was approximately the same in all crosses. The results are shown in Tables 4 and 5. Table 4 shows that a decrease in linkage of unselected markers occurs if the recipient is *dam-3*, and this is exacerbated if the donor is *dam-3* also. Table 5 shows the genetic constitution of the transconjugants. If the recipient is *dam*⁺, then the *thr*⁺ *leu*⁺ *ara*⁺ *pro*⁺ class appears most frequently, whereas if the recipient is *dam-3*, the *thr*⁻ *leu*⁻ *ara*⁻ *pro*⁺ class is predominant. The apparent decrease in linkage, therefore, appears to be due to an increased number of

Table 4. Frequency of unselected markers in crosses between Hfr and F⁻ bacteria

Cross	Frequency of unselected markers			
	<i>thr</i>	<i>ara</i>	<i>leu</i>	<i>pro</i>
1. Hfr <i>dam</i> ⁺ × F ⁻ <i>dam</i> ⁺	51	55	54	100
2. Hfr <i>dam</i> ⁺ × F ⁻ <i>dam-3</i>	23	38	40	100
3. Hfr <i>dam-3</i> × F ⁻ <i>dam</i> ⁺	52	57	58	100
4. Hfr <i>dam-3</i> × F ⁻ <i>dam-3</i>	22	22	26	100

Males and females were mated as described in Materials and Methods and selection made for Pro⁺ transconjugants. The figures represent the pooled data from two separate experiments and 200 recombinants from each cross were scored.

Table 5. Genetic constitution of transconjugants

Hfr	← + + + + + +							
	I	II	III	IV	V			
F ⁻	<i>thr</i>	<i>ara</i>	<i>leu</i>	<i>pro</i>	<i>tsx</i>	<i>str</i>		
Cross-overs in regions	Genotype				Per cent of total recombinants in cross			
	<i>thr</i>	<i>ara</i>	<i>leu</i>	<i>pro</i>	1	2	3	4
I + V	+	+	+	+	43	15	47	13
II + V	-	+	+	+	8	21	9	6
III + V	-	-	+	+	0	3	2	4
IV + V	-	-	-	+	37	52	36	63
I + II + IV + V	+	-	-	+	7	6	4	7
I + III + IV + V	+	+	-	+	1	1	1	2
II + III + IV + V	-	+	-	+	2	1	1	2
I + II + III + V	+	-	+	+	2	1	0	0

The matings were carried out as described in the Materials and Methods and the legend to Table 4. Cross 1 = Hfr *dam*⁺ × F⁻ *dam*⁺; Cross 2 = Hfr *dam*⁺ × F⁻ *dam-3*; Cross 3 = Hfr *dam-3* × F⁻ *dam*⁺; Cross 4 = Hfr *dam-3* × F⁻ *dam-3*.

cross-over events close to the selected marker. Table 5 also shows that the cross-over frequency between two closely linked markers, *ara* and *leu*, is increased two fold if the parents are *dam-3* than if the parents are *dam*⁺. This result was obtained by summing the number of cross-overs in region III in the cross with *dam*⁺ parents (5 cross-overs) and with the *dam-3* parents (11 cross-overs).

4. Discussion

The results of these studies show that the *dam-3* allele confers a hyper-recombination phenotype to the cell. The presence of this mutation leads to an increase in formation of homogenotes from heterogenotes

(Table 2), influences the genetic constitution of trans-conjugants (Tables 4 and 5) and increases the recombination frequency between chromosomal duplications (Konrad, 1976). Surprisingly, there is no increase in chromosome mobilization by F-prime plasmids, which is a *recA* dependent function (Low, 1972).

The molecular basis for the hyper-rec phenotype is not known. Since *dam-3* strains contain more single strand nicks in DNA than the wild type (Marinus and Morris, 1974) it is possible that this leads to an increased level of recombination. Several classes (*dnaB*, *lig*, *dnaS*, *polA*) of hyper-rec mutants isolated by Konrad, are known to cause lesions in DNA.

The formation of homogenotes from heterogenotes offers an alternative method for detecting hyper-rec mutants. We have found that F-*lac* derivatives of *polA12* and *lig-4* bacteria also segregate Lac⁻ clones at a higher frequency than wild type. Such mutants were also shown to be hyper-rec by Konrad (1976).

Acknowledgements. The technical assistance of Diane Cappoli is gratefully acknowledged. This work was supported by U.S. Public Health Service Grants GRS RR 05712 and GM22055. M.G. Marinus is the recipient of a Faculty Research Award (FRA-149) from the American Cancer Society.

References

- Bachmann, B., Low, K.B., Taylor, A.L.: Recalibrated linkage map of *Escherichia coli* K-12. *Bact. Rev.* **40**, 116-167 (1976)
- Clark, A.J., Marguiles, A.D.: Isolation and characterization of recombination deficient mutants of *Escherichia coli* K12. *Proc. nat. Acad. Sci. (Wash.)* **53**, 451-459 (1965)
- Davis, B.D., Mingioli, E.S.: Mutants of *Escherichia coli* requiring methionine or vitamin B12. *J. Bact.* **60**, 17 (1951)
- Jacob, F., Wollman, E.L.: *Sexuality and Genetics of Bacteria*. New York: Academic Press 1961
- Konrad, E.B.: *Escherichia coli* mutations with enhanced frequencies of recombination in a cross between chromosomal duplications. *J. Bact.*, in press
- Low, K.B.: *Escherichia coli* K-12 F-prime factors, old and new. *Bact. Rev.* **36**, 587-607 (1972)
- Luria, S.E., Burrous, J.W.: Hybridization between *Escherichia coli* and *Shigella*. *J. Bact.* **74**, 461-476 (1957)
- Marinus, M.G.: Location of DNA methylation genes on the *Escherichia coli* K-12 genetic map. *Molec. gen. Genet.* **127**, 47-55 (1973)
- Marinus, M.G., Morris, N.R.: Isolation of DNA methylase mutants from *Escherichia coli* K-12. *J. Bact.* **114**, 1143-1150 (1973)
- Marinus, M.G., Morris, N.R.: Biological Function for 6-methyladenine residues in the DNA of *Escherichia coli* K-12. *J. molec. Biol.* **85**, 309-322 (1974)
- Marinus, M.G., Morris, N.R.: Pleiotropic effects of a DNA adenine methylation mutation (*dam-3*) in *Escherichia coli* K-12. *Mutation Res.* **28**, 15-26 (1975)

Communicated by B.A. Bridges

Received and accepted September 10, 1976