

Isolation and Characterization of Dam⁺ Revertants and Suppressor Mutations that Modify Secondary Phenotypes of *dam-3* Strains of *Escherichia coli* K-12

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Summary. Bacteria mutant in the *dam* (DNA adenine methylation) gene and in either *recA* or *recB* or *recC* genes are inviable (Virm⁻ phenotype). From crosses between *dam-3* bacteria and *recA1* or *recB21 recC22* strains, Vrm⁺ recombinants were recovered. Among these recombinants, Dam⁺ revertants were present which did not show the phenotypes normally associated with *dam-3* bacteria. Three classes of indirectly suppressed strains (*dam-3* genotype) were also recovered which showed alterations in the secondary phenotypes normally associated with *dam-3* bacteria. These strains contained a second unlinked mutation in either *mutL* or *mutS* or *sin*. In addition, mutation in either *sbcA* or *sbcB* suppresses the Vrm⁻ phenotype of *dam-3 recB21 recC22* strains.

1. Introduction

The Dam⁻ (DNA adenine methylation deficient) strains of *Escherichia coli* K-12 differ from wild type in that there is no detectable DNA adenine methylase activity in vitro or in vivo and therefore undetectable levels of 6-methyladenine in DNA (Marinus and Morris, 1973; Bale et al., 1979). In addition, *dam* mutant strains differ from wild type in several other respects for which the term secondary phenotypes will be used to distinguish them from the primary Dam⁻ phenotype which is loss of DNA adenine methylase. These secondary phenotypes are designated: AP^s-increased sensitivity to 2-aminopurine (Glickman et al., 1978); UV^s-increased sensitivity to ultra-violet light (Marinus and Morris, 1974, 1975); Vrm⁻-inviability of double mutants containing *dam-3* and *recA*, *recB* or *recC* mutations (Marinus and

Morris, 1974); Flh^H-high frequency of *Flac⁻/lac⁻* (Lac⁻) homogenotes from an *Flac⁺/lac⁻* (Lac⁺) heterozygote (Marinus and Konrad, 1976); Sli^H-high spontaneous induction of λ⁺ prophage (Marinus and Morris, 1975; Goze and Sedgewick, 1978) and Smf^H-high spontaneous mutability (Marinus and Morris, 1974; Glickman et al., 1978; Goze and Sedgewick, 1978).

That these secondary phenotypes are due specifically to mutation in the *dam* gene is uncertain, however, since no Dam⁺ revertants have been isolated and characterized. Bale et al. (1979) have characterized seven independently isolated Dam⁻ strains and found that all seven displayed the secondary phenotypes listed above to varying degrees and have argued that since the secondary traits are common to all Dam⁻ bacteria, they are most probably due to mutation in the *dam* gene.

In this communication we describe a method for isolating Dam⁺ revertants and their subsequent characterization with respect to the primary and secondary phenotypes. We will also describe suppressor mutations which do not suppress the Dam⁻ phenotype but which do suppress some of the secondary phenotypes.

2. Materials and Methods

a) Bacterial Strains

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

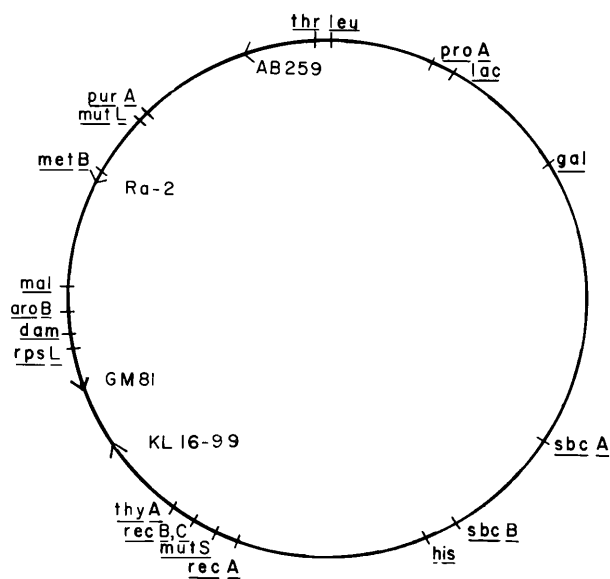
b) Genetic Procedures

Methods for scoring *dam* recombinants by in vitro and in vivo methylation have been described in detail elsewhere (Marinus, 1973; Marinus and Morris, 1973). Sensitivity or resistance to 2-ami-

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Table 1. Genotype of *E. coli* K-12 strains

Strain	Sex	Genotype	Source or reference
AB259	Hfr	<i>thi-1 rel-1</i>	E.A. Adelberg
AT713	F ⁻	<i>argA21 cysC43 lysA22 mtl-2 xyl-7 malA1 rpsL104 (thi-1 supE44)?</i>	B. Bachmann
ES432	F ⁻	<i>ampA73 metB1 leu-6 his-1 mutL25 argG6</i>	E. Siegel
ES1048	Hfr	<i>lacZ(ICR48) trpA9813 mutS3</i>	E. Siegel
GM81	Hfr	<i>dam-3 purF1 metB1 gal-6 lacY1</i> or <i>Z4 tonA2 tsx-1</i>	Marinus and Morris, 1975
GM93	F ⁻	<i>mal-354 tsx-354</i>	Bale et al., 1979
GM100	F ⁻	<i>mal-354 tsx-354 dam-3</i>	Bale et al., 1979
GM112	F ⁻	<i>thr-1 leu-6 proA2 his-4 metB1 lacY1 galK2 ara-14 tsx-33 thi-1 thyA12 deoB6 supE44 rpsL31</i>	Marinus and Konrad, 1976
GM113	F ⁻	As GM112 but <i>dam-3</i>	Marinus and Konrad, 1976
GM160	F ⁻	<i>thr-1 leu-6 proA2 his-4 metB1 lacY1 galK2 ara-14 tsx-33 thi-1 deoB6 supE44 recA1 rpsL31</i>	
GM195	F ⁻	<i>mal-354 tsx-354 dam-3 thyA146</i>	From GM100 by trimethoprim selection
JC4584	F ⁻	<i>recB21 recC22 his-4 galK2 thi-1 endA1</i>	S. Barbour
JC7623	F ⁻	<i>recB21 recC22 sbcB15 thr-1 leu-6 thi-1 lacY1 ara-14 galK2 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31 tsx-33 supE44</i>	A.J. Clark
JC8679	F ⁻	As JC7623 but <i>sbcA23 sbcB⁺</i>	A.J. Clark
KL16-99	Hfr	<i>recA1 (thi-1 drm-3 rel-1)?</i>	K.B. Low
Ra-2	Hfr	<i>mal-28 sfa-4 sfa-5 sup-42</i>	K.B. Low

**Fig. 1.** Genetic map of *E. coli* K-12 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

nopurine (AP) was determined by plating portions of diluted cultures on Brain Heart Infusion agar containing 500 µg AP/ml (Glickman et al., 1978). The procedures for conjugation and transduction have been described by Marinus (1973). Thymine-requiring derivatives were isolated using Trimethoprim as described by Stacey and Simpson (1965). RecA⁺ derivatives of RecA⁻ strains were isolated by selection of methyl methane sulfonate (MMS) resistant colonies on Brain Heart Infusion Agar (20 g/L) containing 0.004% MMS. The RecA⁺ phenotype was verified by showing no co-transducibility of *recA1* with *cysC43* using P1vir lysates prepared from RecA⁺ derivatives.

c) Media and Other Methods

The media for cultivation of bacterial strains and other methods have been described by Bale et al. (1979).

3. Results

a) Inviability of *dam-3 rec⁻* Strains (Vrm⁻ phenotype)

To demonstrate inviability of double mutants, we have used the *recA44*, *recA200*, *recB270* and *recC271*

Table 2. Viable count of *dam-3 rec⁻* strains at 30 C and 42 C

Genotype	Viable count 42 C
	Viable count 30 C
<i>dam⁺ recA200</i>	0.32
<i>dam-3 recA200</i>	6.5×10^{-5}
<i>dam-3 RecA⁺a</i>	1.0
F- <i>dam⁺/dam-3 recA200</i>	0.27
<i>dam-3 recA200 mutL25</i>	0.92
<i>dam⁺ recA44</i>	0.54
<i>dam-3 RecA^a</i>	1.2
<i>dam-3 recA44</i>	2.0×10^{-5}
<i>dam⁺ recB270</i>	0.86
<i>dam-3 RecB⁺a</i>	1.0
<i>dam-3 recB270</i>	2.5×10^{-4}
F- <i>dam⁺/dam-3 recB270</i>	1.0
<i>dam⁺ recC271</i>	0.82
<i>dam-3 RecC⁺a</i>	1.0
<i>dam-3 recC271</i>	2.0×10^{-4}

Stationary phase bacterial cultures were diluted and portions plated on Brain Heart Infusion agar in duplicate. One set of plates was incubated at 30 C, the other at 42 C for 24–48 h before scoring. F-prime derivatives were plated on supplemented minimal agar containing maltose as sole carbon source

^a Denotes that strains are Rec⁺ revertants of Rec⁻ parents

alleles. These mutations produce a Rec⁺ phenotype at 30C and a Rec⁻ phenotype at 42C (Tomizawa and Ogawa, 1972; Lloyd et al., 1974; Hall and Howard-Flanders, 1975). Strains were constructed which contained the *dam-3* allele with or without the *rec⁻* alleles and tested for viability at 30 C and 42 C. The results in Table 1 show that the *dam-3 rec⁻* strains have a low efficiency of plating at 42 C compared to the Dam⁺ *rec⁻* and *dam-3 rec⁺* control strains. F-*dam⁺/dam-3 rec⁻* strains plate with normal efficiency showing that the effect is specific for *dam-3*. Several hundred surviving colonies from the *dam-3 rec⁻* strains grown at 42 C were tested, for recombination proficiency and survival after UV-irradiation, and all were *dam-3 Rec⁺*. The isolation of these Rec⁺ revertants indicate that it is each of the *rec* mutations which cause the lethal effect rather than some other undetected mutation. No Dam⁺ Rec⁻ strains were detected among the survivors able to grow at 42 C.

b) Isolation of Dam⁺ Revertants from Strain GM195

The data in section (a) show that *dam-3 recB270* or *dam-3 recC271* bacteria are inviable at 42 C. We reasoned that since the *dam-3* mutation results in a mutator phenotype (Smf^{HI}) then *dam-3* cultures should contain some Dam⁺ revertants. These should be selected

for by introducing either a *recB⁻* or *recC⁻* non-conditional mutant allele into the population since Dam⁺ *rec⁻* bacteria would survive whereas *dam-3 recB21* or *dam-3 recC22* bacteria would not. Accordingly, a *dam-3 ThyA⁻* (GM195) strain was transduced with a P1vir lysate prepared on strain JC4584 (*recB21 recC22*) and Thy⁺ recombinants selected. Six transductants out of 100 were as sensitive to ultraviolet (UV) light as an authentic *recC22* strain. Five of these transductants were Dam⁺ as determined by in vitro or in vivo methylation and one was Dam⁻. From one of the Dam⁺ *rec⁻* strains a thymine-requiring derivative was isolated and this was transduced with a P1vir lysate prepared on GM28 (prototrophic) and selection made for Thy⁺, and a Rec⁺ derivative (GM170) was obtained. The primary and secondary phenotypes of GM170 were then compared to GM100, the original *dam-3* parent, and its isogenic wild type strain GM93. The results in Table 3 show that GM170 has the primary Dam⁺ phenotype of GM93 together with all the wild type secondary phenotypes: AP^R UV^R Vrm⁺ Flh^N Sli^N and Smf^N (where the superscript N denotes the wild type level).

c) Isolation of a Dam⁺ Revertant from Strain GM113

The data in section (a) show that *dam-3 recA200* and *dam-3 recA44* strains are inviable. For the reason described in the section above, Hfr KL16–99 (*recA1*) was mated with GM113 (*dam-3 thyA12*) for 60 min and Thy⁺ Str^R recombinants were isolated. The cells were mated for only 60 min to prevent transfer of the *dam⁺* gene from the donor, to the recipient. From 1,400 Thy⁺ recombinants, 12 strains were recovered which were as sensitive to UV-irradiation as an authentic *recA1* strain. One of these (GM171) was found to be Dam⁺ by in vitro or in vivo methylation assays. An analysis (Table 3) of a methyl methane sulfonate resistant (i.e., RecA⁺) derivative (GM172) of GM171, shows that it is identical to wild type with respect to its secondary phenotypes compared to strain GM113 (*dam-3*).

The *dam* gene is known to be 80% co-transducible with *trpS* and 90% co-transducible with *aroB* (Marinus, 1973). A P1vir lysate was prepared from GM172 and used to transduce *trpS10330* and *aroB351* recipients to Trp⁺ and AroB⁺ respectively. None (0/20) of the Trp⁺ or AroB⁺ transductants had a Dam⁻ phenotype. These results exclude the possibility that GM172 contains an unlinked or distantly linked mutation suppressing *dam-3*. It cannot be determined whether the transconjugant GM171 inherited *dam⁺* following transfer from the donor strain or following backmutation of *dam-3* in one of the

Table 3. Phenotypic characterization of wild type and mutant strains

Strain	Pertinent Genotype	Mole % 6-meA ^a	AP ^b	Mutant frequency ^c	Recombination Pro-ficiency ^d	F- <i>lac</i> homogenote frequency ^e	PFU of λ /cell ^f	Frequency of clear plaques ^g	Survival after UV ^h
GM93	wild	2.0	R	2×10^{-8}	n.d. ⁱ	1	1.2×10^{-3}	$< 10^{-3}$	36
GM100	<i>dam-3</i>	≤ 0.06	S	1.7×10^{-6}	n.d.	15	2.4×10^{-2}	1.2×10^{-3}	18
GM170	<i>dam</i> ⁺ ^j	1.9	R	1×10^{-8}	n.d.	1	6×10^{-4}	$< 10^{-3}$	40
GM112	wild	2.2	R	1×10^{-8}	20.2	1	2.5×10^{-3}	$< 10^{-3}$	37
GM113	<i>dam-3</i>	≤ 0.06	S	5.6×10^{-7}	17.1	18	1.2×10^{-2}	3.0×10^{-3}	6
GM172	<i>Dam</i> ⁺ ^j	1.9	R	1×10^{-8}	19.5	1	1.5×10^{-3}	$< 10^{-3}$	34
GM160	<i>recA1</i>	2.1	R	5×10^{-9}	4.3×10^{-3}	< 0.01	4.8×10^{-6}	0.93	5
GM158	<i>dam-3 recA1 mutL451</i>	≤ 0.06	R	1.3×10^{-4}	3.6×10^{-2}	< 0.01	3.8×10^{-4}	0.98	8
GM150	<i>dam-3 mutL451</i>	≤ 0.06	R	1.1×10^{-4}	19.4	2	2.7×10^{-3}	2.7×10^{-2}	38
GM168	<i>dam-3 recA1 mutS453</i>	≤ 0.06	R	7.0×10^{-5}	4.5×10^{-3}	< 0.01	1.3×10^{-5}	1.0	3
GM169	<i>dam-3 mutS453</i>	≤ 0.06	R	9.1×10^{-5}	16.6	5	2.3×10^{-2}	1.5×10^{-3}	28
GM178	<i>dam-3 recA1 sin-1</i>	≤ 0.06	S	2.6×10^{-6}	$< 1 \times 10^{-3}$	< 0.01	2.5×10^{-4}	0.98	5
GM179	<i>dam-3 sin-1</i>	≤ 0.06	S	1.1×10^{-7}	13.0	11	4.4×10^{-2}	$< 10^{-3}$	20
JC7623	<i>recB21 recC22 sbcB15</i>	2.0	R	1×10^{-8}	8.0	1	2.7×10^{-3}	$< 10^{-3}$	32
GM216	<i>recB21 recC22 sbcB15 dam-3</i>	≤ 0.06	S	4.210^{-6}	8.3	11	1.8×10^{-3}	$< 10^{-3}$	1.8
JC8679	<i>recB21 recC22 sbcA23</i>	1.8	R	2×10^{-8}	13.8	2.5	2.8×10^{-3}	$< 10^{-3}$	36
GM163	<i>recB21 recC22 sbcA23 dam-3</i>	≤ 0.06	S	1.2×10^{-7}	2.0	14	4.8×10^{-2}	2.7×10^{-3}	18

^a Mole % refers to the number of 6-meA residues per 100 adenine residues

^b S=growth inhibited by AP; R=no inhibition of growth

^c The number of rifampicin resistant mutants per number of sensitive cells plated

^d Recombination proficiency refers to the number of Pro⁺ Str^R recombinants per 100 Hfr AB259 donors under standard conditions

^e The percentage of clones which are Lac⁻ on MacConkey lactose medium and permissive for phage λ . One hundred-Two hundred cells were inoculated into 1 ml brain heart broth and grown overnight at 37 C without aeration before plating

^f The number of plaque forming units (PFU) of phage per bacterium in the logarithmic ($1-2 \times 10^8$ cells/ml) phase of growth of a lysogenic strain

^g The number of clear plaque formers of phage per total number of plaques

^h The per cent survival after UV-irradiation with 20J/m² (RecA⁺) or 0.3J/m² (RecA⁻)

ⁱ n.d.=not done

^j Indicates back mutation from *dam-3* to *dam*⁺

recipient cells. Thus the conjugation test cannot be used to prove that *dam-3* is backmutable.

d) Class I Suppressed Strains

The data in section (c) showed that 12 Thy⁺UV^s recombinants were obtained after mating Hfr KL16-99 (*recA1*) with GM113 (*dam-3 thyA12*). One of these recombinants (GM172) was *dam*⁺, and eleven were *dam-3*. Since *dam-3 recA1* strains should be inviable (Vrm⁻ phenotype) it was reasoned that these eleven *dam-3 recA1* strains are viable due to suppression of the Vrm⁻ phenotype by another mutation. For clarity, these eleven strains are separated into classes based on genetic and phenotypic characteristics. Only one member of each class will be described since strains within each class have identical pheno-

types and genotypes. The effects of various suppressor mutations on primary and secondary phenotypes are summarized in Table 4.

There are two mutant strains in class I and a description of GM158 (*dam-3 recA1 mutL451*) and its RecA⁺ derivative, GM159 is given in Table 3. GM158 is UV^s Rec⁻ but slightly more Rec⁺ than the *dam*⁺ *recA1* comparison strain GM160. This is shown by the ten fold increase in recombination frequency when the strains are mated with AB259 (Table 3). An analysis of the Pro⁺ Str^R transconjugants showed that they were all UV^s from the AB259 \times GM160 cross, but were all UV^R from the AB259 \times GM158 cross. This suggests that either a large number of Rec⁺ revertants are present in cultures of GM158 or that there is a high frequency of inheriting *recA*⁺ from the donor. The problem is resolved by looking at the phenotype of GM150,

Table 4. The effect of suppressor mutations on primary and secondary phenotypes of *dam-3* bacteria

Pheno-type	None	Locus of suppressor mutation				
		<i>mutL</i>	<i>mutS</i>	<i>sin</i>	<i>sbcA</i>	<i>sbcB</i>
Dam	—	—	—	—	—	—
AP	S	R	R	S	S	S
UV	S	R	R	S	S	S
Vrm	—	+ ¹	+ ²	+ ¹	+ ³	+ ³
Flh	H	N	N	H	H	H
Sli	H	N	H	H	H	H
Smf	H	HH	HH	H	H	H

S=sensitive, R=resistant, H=high, HH=very high, N=normal; Vrm⁻ denotes inability to suppress lethality of Dam⁻ Rec⁻ strains. Vrm⁺ denotes suppression of Dam⁻ Rec⁻ lethality. The superscript numbers above Vrm⁺ denote that it was (1) Tested only with *recA1* (2) Tested with *recA1* and *recB21 recC22* and (3) Tested only with *recB21 recC22*

a UV^R Rec⁺ derivative of GM158, which has a very high spontaneous mutation frequency compared to strain GM113 (*dam-3*). This leads to the conclusion that cultures of GM158 contain large numbers of RecA⁺ revertants and suggests that the suppressor mutation is itself a mutator.

Genetic mapping revealed that in strain GM150, the suppressor mutation, which shows a mutator phenotype and resistance to AP, is located between the origins of Hfr Ra2 and AB259, and further mapping with P1*vir* using AP resistance as a selective marker showed that the suppressor mutation is 80–90% co-transducible with *purA*. In addition to GM158, there is another class I strain (GM176), and it was also found to have a suppressor mutation, conferring a mutator phenotype and AP resistance, which is 80–90% co-transducible with *purA*. Siegel and Ivers (1975) have described a mutator gene, *mutL*, which is co-transducible with *purA* and this suggests that GM158 and GM176 may have a mutated *mutL* allele. To test if *mutL*⁻ can suppress *dam-3* RecA⁻ lethality, *mutL23* (Siegel and Ivers, 1975) was transduced into strain GM84 (*dam-3 recA200*; see section (a)), using the co-transducible *ampA73* marker. The resultant derivative, GM165, is Vrm⁺, Sfm^{HH} and Dam⁻. The superscript HH denotes a very high mutation frequency. The properties of GM165 are consistent with the hypothesis that *mutL25* suppresses some of the secondary phenotypes of *dam-3* bacteria.

Compared to GM113 (*dam-3*), strain GM150- (*dam-3 mutL451*) has the secondary phenotypes expected of a Dam⁺ strain with the exception of a very high spontaneous mutation frequency (Table 3). These results are consistent with the hypothesis that *mutL451* suppresses, with the exception of sponta-

neous mutation frequency, the secondary phenotypes of *dam-3* strains.

It seems probable therefore, that the mutations causing AP resistance and mutator phenotype in Class I strains are located in the *mutL* gene and the two suppressor mutations in these strains have tentatively been designated *mutL451* and *mutL452*.

e) Class II Suppressed Strains

GM168, (*dam-3 recA1 mutS462*) isolated from the same cross that produced the Class I mutant GM158, is phenotypically similar to GM158 in UV sensitivity, recombination deficiency and AP resistance. A Rec⁺ revertant (GM169) showed the very high spontaneous mutation frequency also shown by the Rec⁺ revertants of the Class I mutant. Genetic mapping revealed, however, that the suppressor mutation conferring AP resistance and very high spontaneous mutation frequency is not near *purA* but is 50% co-transducible with *cysC*⁺. The data suggest that the suppressor mutation in GM168 may be in the *mutS* gene since *mutS* maps close to *cysC* (Cox et al., 1972). F-prime heterogenotes (*F-thy A-cysC-mutS*) of GM169 do not display a high spontaneous mutation frequency showing that the suppressor mutation is recessive.

In section (b), the isolation of five Dam⁺ revertants from GM195 was described and, in addition, the isolation of one strain (GM173) which was *dam-3* but Vrm⁺. A Rec⁺ derivative of this strain is identical in phenotype to GM169 (Data not shown) and the mutation causing AP resistance is 60% co-transducible with *cysC43*. This suggests that the suppressor mutation in GM173 may also be in the *mutS* gene. The suppressor mutation in this strain has been tentatively designated *mutS462* and that in GM168 as *mutS453*. The secondary phenotypes of class II strains are qualitatively similar to those of class I (Table 3) suggesting that mutation in the *mutS* gene can suppress some of the secondary phenotypes of *dam-3* strains.

f) Class III Strains

The eight remaining Dam⁻ Rec⁻ Vrm⁺ strains have been included in class III and are similar in phenotype. The phenotypes of RecA⁺ derivatives of these eight strains are also similar. It has not been possible to map the location of the suppressor mutation, designated *sin*, in Class III strains because of the absence of a selective phenotypic marker. The phenotypes of a selected Dam⁻ Rec⁻ strain (GM178) and its RecA⁺

derivative (GM179) are shown in Table 3. The phenotypes of GM179 and GM113 are virtually identical except for differences which may not be significant in sensitivity to UV irradiation and frequency of clear plaque mutants among lambda phage, spontaneously produced from a λ^+ lysogen. An interesting difference in Sfm^H phenotype is shown between GM178 and GM179 in that the mutant frequency is 23 times higher in GM179 than GM178.

g) Suppression of Vrm⁻ Phenotype of dam-3 Strains by sbcA23 and sbcB15

The phenotype of *recB21 recC22* strains can be suppressed by second site mutations in either *sbcA* (Barbour et al., 1970) or *sbcB* (Kushner et al., 1971). It was of interest to determine if these mutations could suppress the inviability of *dam-3 recB21 recC22* strains. The *dam-3 recB21 recC22 sbc⁻* strains were constructed by introducing the *dam-3* allele by conjugation, using Hfr GM81, into strains JC8679 (*recB21 recB22 sbcA23*) and JC7623 (*recB21 recC22 sbcB15*) by selection for Xyl⁺ Dam⁻ recombinants. From these crosses, we isolated strain GM216 (*dam-3 recB21 recC22 sbc15*) and GM163 (*dam-3 recB21 recC22 sbcA23*). Both these strains were Dam⁻ as determined by in vitro and in vivo methylation. P1vir lysates of GM98 and GM163, when used to transduce *thyA12* recipients to Thy⁺ resulted in the recovery of Rec⁻ derivatives at a frequency of 60–70%. The phenotypes of strains GM216 and GM163 differ in only the Vrm phenotype compared to the *dam-3* strain, GM113 (Table 3), suggesting that mutation in *sbcA* or *sbcB* can suppress the Vrm⁻ phenotype of *dam-3 recB21 recC22* strains.

4. Discussion

A prime objective of this investigation was to determine if the secondary phenotypes of *dam-3* bacteria are due specifically to mutation in the *dam* gene. The data in Table 3 for the Dam⁺ revertants, leads to the conclusion that they are due specifically to mutation in the *dam* gene. In addition, we have characterized strains in which mutations in *mutL*, *mutS*, *sin*, *sbcA*, *sbcB* genes acting indirectly modify the secondary phenotypes of *dam-3* mutants but not the primary phenotype (i.e. DNA methylation). The effects of these indirect suppressors are summarized in Table 4. It should be noted that the assignment of *MutS⁻* and *MutL⁻* is tentative and awaits complementation tests as a rigorous genetic proof. In a related study,

B.W. Glickman (personal communication) has isolated AP resistant strains from Dam⁻ bacteria and found that these are *dam⁻ mutL⁻*, *dam⁻ mutS⁻* and *dam⁻ mutR⁻* double mutants.

The secondary phenotypes of *dam-3* bacteria are suggestive of a role for the *dam* gene product in a DNA repair pathway. The repair pathway involved may be that which recognizes and removes base pair mismatches in DNA. Rydberg (1977, 1978) has shown that the *mutL*, *mutS*, *mutR* and *uvrE* gene products are required for repair of mismatched bases in phage λ heteroduplexes. Ryokowski, Pukkila, Radman, Wagner and Meselson (personal communication) have proposed that DNA adenine methylation serves as the basis for strand discrimination in the repair of mismatched bases in phage λ heteroduplexes. The observations in this communication that mutation in *mutL* and *mutS* genes indirectly suppress most of the secondary phenotypes of *dam-3* mutants are compatible with the proposed model of Ryokowski et al. In *dam-3 Mut⁻* strains, mismatch repair is reduced and leads to an observed increase in mutation rate (Sfm^H). In *dam-3 Mut⁺* strains, mismatch repair is operative but mistakes occur (Sfm^H) since the repair system cannot discriminate between parental and daughter strands. The secondary phenotypes of *dam-3* strains presumably are consequences of DNA repair and would not be observed in the absence of such repair. The secondary phenotypes in *dam-3 MutL⁻* and, except for the Sli phenotype, in *dam-3 MutS⁻* are in agreement with this idea (Table 4).

The existence of the *sbcA*, *sbcB* and *sin* suppressor mutations are not readily explained by the above model. Either these gene products are indirectly involved in mismatch repair or the *dam* gene product is also involved in some other cellular process involving these gene products.

The hypothesis that some mutations suppressing the Vrm⁻ phenotype lie in a gene called *sin* is in doubt since it has not been possible to locate it on the genetic map. An alternative explanation for the existence of class III Dam⁻ RecA⁻ strains would be that the *recA1* gene product in these strains is altered in such a way to allow survival of *dam-3* bacteria but which still is defective in repair and recombination. Experiments are in progress to test this possibility.

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