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Viability of *Escherichia coli* K-12 DNA adenine methylase (*dam*) mutants requires increased expression of specific genes in the SOS regulon

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Summary. We have examined the level of expression of the SOS regulon in cells lacking DNA adenine methylase activity (dam^-). Mu*d* (Ap, *lac*) fusions to several SOS operons (*recA, lexA, uvrA, uvrB, uvrD, sulA, dinD* and *dinF*) were found to express higher levels of β-galactosidase in dam^- strains than in isogenic dam^+ strains. The attempted construction of dam^- strains that were also mutant in one of several SOS genes indicated that the viability of methylase-deficient strains correlates with the inactivation of the SOS repressor (LexA protein). Consistent with this, the wild-type functions of two LexA-repressed genes (*recA* and *ruv*) appear to be required for dam^- strain viability.

Introduction

The *dam* gene of *Escherichia coli* K-12 codes for a DNA adenine methylase which methylates -GATC- sequences in double-stranded DNA (for review see Marinus 1984). The primary phenotypes associated with mutations in this gene are the lack of detectable DNA adenine methylase activity in vivo or in vitro and the lack of detectable levels of 6-methyladenine in the DNA of *dam* strains (Marinus and Morris 1974; Bale et al. 1979). Secondary phenotypes include increased sensitivity to DNA-damaging treatments (ultraviolet light, 2-aminopurine, etc.), a hyper-rec phenotype, increased spontaneous mutability, increased spontaneous induction of prophages and increased precise excision of certain transposons (Marinus and Morris 1974; Marinus and Morris 1975; Marinus and Konrad 1976; Glickman et al. 1978; Goze and Sedgewick 1978; Lundblad and Kleckner 1982; Arraj and Marinus 1983; Marinus et al. 1984). DNA adenine methylase mutants have a greater frequency of single-stranded breaks in their DNA; this phenomenon is amplified in *dam lig* or *dam polA* double-mutants (Marinus and Morris 1974; Marinus and Morris 1975). Most dam^- phenotypes are suppressed by $mutH^-$, $mutL^-$ or $mutS^-$, and *dam* mutants are inviable in combination with *recA*, *lexA*(Ind$^-$), *recB, recC* or *polA* mutations (Glickman and Radman 1980; Marinus and Morris 1974; Marinus and Morris 1975; McGraw and Marinus 1980).

The inviability of *dam recA* and *dam lexA*(Ind$^-$) double mutants suggests a requirement for expression of the SOS regulon in dam^- strains. The SOS response of *E. coli* is marked by drastic alterations in cellular physiology following treatments which damage DNA or inhibit DNA replication (for reviews see Witkin 1976; Little and Mount 1982; Walker 1984). These physiological changes are the consequence of the derepression of at least seventeen unlinked operons which are negatively regulated by LexA repressor (Brent and Ptashne 1981; Little et al. 1981), collectively called the SOS regulon. Treatments that induce the SOS response do so by activating RecA protein. This activated form of RecA facilitates the proteolytic cleavage of LexA repressor, which results in derepression of the regulon (Little et al. 1980; Horii et al. 1981; Little 1983). Two alleles of *lexA*, *lexA1* and *lexA3*, were shown to be inviable in combination with dam^- (Marinus and Morris 1975; unpublished observations). Both of these are members of the *lexA* (Ind$^-$) class, the products of which do not undergo cleavage in induced cells (Little et al. 1980; Little 1983; Peterson and Mount, unpublished experiments). Certain *recA* mutants, such as *recA1* or *recA200*(Ts) at nonpermissive temperature, also block induction of SOS functions because they are not capable of promoting LexA repressor cleavage; they too are inviable with dam^- (Marinus and Morris 1974; Marinus and Morris 1975; Roberts et al. 1978; McGraw and Marinus 1980; Hickson et al. 1981). The inability to construct dam^- strains with *recA* or *lexA*(Ind$^-$) mutations suggested that expression of the SOS response is required in strains deficient in DNA adenine methylase. We have tested this hypothesis in two ways: i) by attempting to construct dam^- strains with one of several mutant SOS genes, ii) by assaying the expression of several SOS genes in dam^- and dam^+ isogens.

In this paper we demonstrate that one of the properties of dam^- strains is an increased expression of some genes of the SOS regulon and that these increases are required for dam^- viability. Our studies of SOS mutant strains indicate that the products of at least two genes repressed by LexA protein are required. These results indicate that *E. coli* is capable of responding to some alterations in DNA metabolism by establishing an increased basal level of SOS expression.

Materials and methods

Bacterial and phage strains. The *Escherichia coli* K-12 strains used in this study are listed in Table 1. P1 phage transductions using P1*vir* were performed by a standard genetic method (Miller 1972) except that infected cells were grown non-selectively for two hours in L broth prior to antibiotic selection. Mu*d* (Ap, *lac*) fusions, except for *uvrD*, were converted to stable Mu*d*XCam derivatives by the pro-

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Table 1. *Escherichia coli* K-12 strains

Strain	Genotype	Source
GW1000	*recA441 sulA211 lacΔ* (U169) *thr-1 leuB-6 his-4 argE3 relA galK2 rpsL31 supE44*	(Kenyon and Walker 1980)
GW1060	as GW1000 but *uvrA215*::Mu*d*(Ap, *lac*)	(Kenyon and Walker 1980)
GW1070	as GW1000 but *dinF1*::Mu*d*(Ap, *lac*)	(Kenyon and Walker 1980)
GW1300	as GW1000 but *uvrB125*::Mu*d*(Ap, *lac*)	G.C. Walker
KP210	as GW1000 but *recA*$^+$ *srlC*::Tn*10* *dam-4 cysG*::Tn*5* λ*cI*(Ind$^-$) *recA*::*lac*	This paper[a, b]
KP264	as KP210 but *dam*$^+$	This paper
KP266	as KP210 but λ*cI*(Ind$^-$) *lexA*::*lac* *dam*$^+$	This paper
KP267	as KP266 but *dam-4*	This paper
KP211	as GW1070 but *dam-4 cysG*::Tn*5* *recA*$^+$ *srl zja505*::Tn*10* *dinF1*::Mu*d*XCam	This paper[a, b, c]
KP212	as KP211 but *dam*$^+$	This paper
NO22	F′ *lac relA thi zja505*::*Tn10* *sulA*::Mu*d*(Ap, *lac*)(Mu$^+$)	Nina Ossanna
KP186	as NO22 but *dam-4 cysG*::Tn*5*	This paper[b]
KP129	as GW1060 but *recA*$^+$ *srl* Tet^s *uvrA215*::Mu*d*XCam	This paper[a]
KP187	as KP129 but *dam-4 cysG*::Tn*5*	This paper[b]
KP132	as GW1300 but *recA*$^+$ *srl* Tet^s *uvrB125*::Mu*d*XCam	This paper[a]
KP188	as KP132 but *dam-4 cysG*::Tn*5*	This paper[b]
KP205	as NO22 but *malB45 lexA41* *cysG*::Tn*5*	This paper[d]
KP206	as KP205 but *dam-4*	This paper[b]
ES1597	*uvrD273*::Mu*d*(Ap, *lac*) *araD139* *lacΔ* (U169) *rpsL31 thi*	(Siegel 1983)
GM2164	as ES1597 but *dam-13*::Tn*9*	This paper[e]
NO56	as NO22 but *cps3 sulA*::Mu*d*XCam *malF55*::Tn*5* Tet^s	Nina Ossanna
KP182	as NO56 but *lexA41 zja505*::Tn*10* *malB45*	This paper[d]
KP183	as NO56 but *zja505*::Tn*10*	This paper[c]

[a] The donor strain for *recA*$^+$ *srlC*::Tn*10*, JL407, was provided by J.W. Little
[b] The donor strain for *dam-4 cysG*::Tn*5* was GM1737 (Arraj and Marinus 1983)
[c] *zja505*::Tn*10* was from KP84 *lexA41 zja505*::Tn*10 malB45*
[d] *lexA41 malB45* was from KP84 *lexA41 zja505*::Tn*10 malB45*
[e] The donor strain for *dam-13*::Tn9 was JC7623 *dam-13*::Tn9 (Marinus et al. 1983)

cedure of Baker et al. (1983). Tetracycline-sensitive derivatives of Tet^r strains were selected on Bochner plates as modified by Maloy and Nunn (1981). Cells were lysogenized with lambda phage bearing *lac* fusions by infecting log-phase cells in liquid at a multiplicity of infection of 0.1; aliquots were plated on MacConkey lactose agar for identification of Lac$^+$ colonies. Relative β-galactosidase activity was used to insure that a monolysogen was used in subsequent assays. λ*cI*(Ind$^-$) *recA*::*lac* (obtained from J.W. Little) is a derivative of λGW271 (Weisseman et al. 1984) and carries an operon fusion of the *recA* operator-promoter region to the *lac* operon.

The phage λ*cI*(Ind$^-$) *lexA*::*lac* was constructed by insertion of a 118 basepair *lexA*op fragment into the promoter cloning vector, λRS205 (Bertrand et al. 1984). Because this vector was designed for the cloning of *Eco*RI/*Sal*I fragments it was necessary to introduce a *Sal*I site near the beginning of the *lexA* coding sequence. This was done by cutting the *lexA*$^+$ plasmid, pJWL42 (Markham et al. 1981), with *Eco*RI and *Bcl*I, and isolating a 148 basepair *lexA*op-bearing fragment. This fragment was cut with *Hae*III and the resulting 118 basepair fragment was ligated with pBR322 (Sutcliffe et al. 1978) that had been sequentially treated with *Sal*I, Klenow fragment of PolI (for conditions see Wertman et al. 1984) and *Eco*RI. This procedure regenerated the *Sal*I site and provided a plasmid (pKW2) that served as a source of the *Eco*RI-*Sal*I *lexA*op fragment that was cloned into λRS205. The resultant λ*cI857 lexA*::*lac* phage was crossed with λ*cI*(Ind$^-$) by a standard method (Davis et al. 1980) and turbid, Lac$^+$ plaques were identified at 42° C on lambda Xgal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) plates.

Media. L broth contained 10 g Bacto-tryptone (Difco), 5 g yeast-extract (Difco), 10 g NaCl per liter of water, pH 7.0. 1×A broth was as described by Miller (1972) but supplemented with 0.5% vitamin-free Casamino acids (Difco), 0.004% uracil and 0.005% cysteine. L plates were L broth with 1.5% agar and LB plates were as L broth with 0.2% glucose and 2% agar. Lambda Xgal plates contained, per l of water, 10 g Bacto-tryptone, 5 g NaCl, 10 g agar and 50 μg/ml Xgal. Media were supplemented, when required, with antibiotics at concentrations of 20 μg/ml (chloramphenicol), 50 μg/ml (ampicillin), 25 μg/ml (tetracycline), 80 μg/ml (kanamycin), or 0.25 μg/ml (mitomycin-C).

Inviable combinations. The inability to construct *dam lexA3* (Ind$^-$) and *dam recA1* recombinants has been dealt with in detail by Marinus and Morris (1975) and McGraw and Marinus (1980), respectively. We attempted to construct a *dam lexA51* (Def) *sulA211 recA56* recombinant by mating a *recA56 srl*::Tn*10* HfrPO45 with a *dam lexA51* (Def) *sulA211 rpsL31 his-4* recipient. The recombination frequency for tetracycline and streptomcyin resistant recombinants was one hundred to one thousand fold less than with the isogenic F$^-$ *dam*$^+$ strain. None of the tetracycline resistant recombinants were *recA*$^-$ as determined by sensitivity to ultraviolet irradiation. His$^+$ recombinants were formed at low frequency indicating that the Hfr transferred this marker and that it was integrated in a small fraction of the population. The construction of a *dam lexA3* (Ind$^-$) *recAo98 sulA211* recombinant was attempted by mating an Hfr which transfers *dam-13*::*Tn9* as an early marker with a *lexA3* (Ind$^-$) *recAo98 sulA211 rpsL31* recipient. The recombination frequency for chloramphenicol – streptomycin resistant recombinants was about one thousand fold less than with an isogenic *lexA*$^+$ recipient. Most of these recombinants were *dam*$^+$. Those which were *dam*$^-$ had simultaneously lost the *lexA3* (Ind$^-$) marker. The existence of this latter class shows that transfer from the donor to recipient had occurred. Experiments similar to these were used to demonstrate that a *dam-13*::Tn9 *recJ77* recombinant could not be isolated from a mating between a *dam-13*::Tn9 Hfr and a *recJ77* recipient. The *dam-4* allele

and *cysG*::Tn*5* are 45–58% linked in P1-transductional crosses when selecting for kanamycin resistance in ruv^+ strains. The linkage value was reduced to zero (0/144) when a *ruv* mutant strain was employed as a recipient.

β-galactosidase assays. β-galactosidase activity was measured according to Miller (1972). Overnight cultures of cells grown in 1 × A broth plus appropriate antibiotics were diluted into fresh 1 × A broth minus antibiotics and grown at 37° C to 5×10^7 cells/ml. Cultures were split and differentially treated or not, as indicated, and further incubated for four hours at which time β-galactosidase activity was determined. All values are the average of duplicate experiments (two samples per experiment).

Results and discussion

Inviability of dam^- *lexA*(Ind$^-$) *strains*

Three classes of *lexA* mutations have been described, *lexA* (Ind$^-$), *LexA*(Def) and *lexA*(Ts) (Little and Mount 1982). *LexA*(Ind$^-$) repressors are resistant to RecA protein-mediated proteolysis; SOS operons are not derepressed following inducing treatments. *lexA*(Def) strains make a protein that no longer functions as a repressor; therefore the SOS functions are constitutively expressed. *lexA*(Ts) mutants produce a repressor that is thermally inactivated resulting in derepression of SOS functions at nonpermissive temperature. The viability of dam^- *lexA*(Def) strains and inviability of dam^- *lexA*(Ind$^-$) strains has been demonstrated (Marinus and Morris 1975; see Table 2). These data suggest that unconditional LexA repressor function results in lethality in dam^- strains. This hypothesis was tested using a *lexA*(Ts) allele.

The *lexA41* (formerly *tsl-1*) allele encodes a repressor that appears to be temperature sensitive (*lexA*(Ts); Mount et al. 1973). It was isolated as a UV-resistant derivative of the *lexA3*(Ind$^-$) allele (Mount et al. 1973). *lexA41* is partially *lexA*(Def) at 42° C and *lexA*(Ind$^-$) at 30° C as inferred from its origin and from the data shown in Table 3. LexA41 protein does not repress *sulA* efficiently at 42° C, but has the ability to repress *sulA* at 30° C. The DNA-damaging agent mitomycin-C (MMC) does not induce *sulA* in a *lexA41* background as it does in a $lexA^+$ strain, revealing the *lexA*(Ind$^-$) character of *lexA41* at permissive temperature. One would predict then that *lexA41* dam^- strains should survive at 42° C but not at 30° C, and that *lexA41* dam^+ strains should be viable at either temperature. This is indeed the case (see Table 4). These results clearly demonstrate that the viability of dam^- strains correlates with LexA repressor inactivation.

Inviability of dam^- $recA^-$ *strains*

The necessity for $recA^+$ function in dam^- strains could be explained solely by the need to inactive LexA repressor. However, inactivation of LexA protein via a *lexA*(Def) mutation is not adequate to allow survival of a *recA dam* double mutant (see Table 2) suggesting a requirement for an additional $recA^+$ function(s). We infer that RecA protein is necessary first in its regulatory capacity for LexA repressor proteolysis and second, in one or more of the other $recA^+$ activities (recombination or mutagenesis). *recA430* is incapable of cleaving λ repressor, has reduced ability to promote cleavage of LexA repressor (Roberts and Roberts 1981; Kawashima et al. 1984; Rebollo et al. 1984; Peterson and Mount, unpublished data), is recombination proficient (Morand et al. 1977), and is unable to perform its role in inducible mutagenesis (Blanco et al. 1975; Ennis et al. 1985). Since *recA430* is almost fully competent in recombi-

Table 2. Viability of dam^- derivatives with mutant alleles of the damage-inducible (SOS) repair system

Inviable combination	Viable combination
dam recA1	*dam uvrA6; dam uvrB5;*
dam lexA3(Ind$^-$)	*dam uvrC34;* *dam uvrD273*::Mu*d*(Ap, *lac*);
dam lexA51(Def) *sulA211 recA56*	*dam sulA211;* *dam umuC121*::Tn*5;*
dam lexA3(Ind$^-$) *recAo98 sulA211*	*dam dinA1*::Mu*d*(Ap, *lac*);
dam ruv-61	*dam dinB1*::Mu*d*(Ap, *lac*);
dam recJ77	*dam dinD1*::Mu*d*(Ap, *lac*);
	dam dinF1::Mu*d*(Ap, *lac*);
	dam recF143; dam himA81;
	dam recAo98 sulA211;
	dam recA441(*tif-1*);
	dam recA453(*tif-1 zab-53*);
	dam recA430(*lexB30*)
	dam lexA51(Def) *recA441* (*tif-1*) *sulA211*

Strains were constructed by introducing a *dam* mutant allele (*dam-3, -4, -12*::Mu*d* or *-13*::Tn*9*) into recipient strains except for crosses involving $recA^-$ in which $recA^-$ donors and dam^- recipients were employed. Inviable combinations are those in which the desired strain was not recovered (see Materials and methods)

Table 3. β-galactosidase activity of $lexA^+$ and *lexA41 sulA*::Mu*d*XCam at 30°, 42° C and 30° + Mitomycin-C

Strain	*lexA* allele	β-galactosidase activity (Miller units)		
		30° C	42° C	30° C + MMC
KP183	+	53	44	781
KP182	*41*	43	245	45

Overnight cultures were diluted in fresh 1 × A broth and grown at 30° C to 5×10^7 cells/ml, at which time they were split three ways. One part was shifted to 42° C, to the second part was added mitomycin-C (final concentration, 0.25 μg/ml) and the third part was left untreated. Incubation was continued for 4 h, at which time β-galactosidase activity was assayed

Table 4. Efficiency of plating (EOP) for *lexA41* dam^- and *lexA41* dam^+ strains

Strain	*dam* allele	Colony-forming (units per ml)		EOP (30° C/42° C)
		30° C	42° C	
KP205	+	2.0×10^8	2.2×10^8	0.91
KP206	*4*	3.8×10^3	5.1×10^7	7.5×10^{-5}

Overnight 42° C L broth cultures were diluted in fresh L broth and grown to approximately 2×10^8 cells/ml at 42° C. Appropriate dilutions were made in L broth and cells were plated on L plates in duplicate. Two sets were made for each strain; one set was incubated overnight at 42° C, the other at 30° C

Table 5. *β*-galactosidase activity in dam^+ and dam^- derivatives of *E. coli* K-12 SOSop (operator-promoter)::*lac* fusion strains

Strain	*lac* fusion	*dam* allele	*β*-galactosidase activity (Miller units)		$\frac{dam^-}{dam^+}$	$\frac{+mmc}{-mmc}$
			−MMC	+MMC		
KP264	*recA*	+	1218	6080	3.0	5.0
KP210	*recA*	*4*	3646	ND		
KP266	*lexA*	+	340	608	1.7	1.8
KP267	*lexA*	*4*	583	ND		
NO22	*sulA*	+	131	4117	5.9	31
KP186	*sulA*	*4*	769	ND		
KP129	*uvrA*	+	87	311	2.2	3.6
KP187	*uvrA*	*4*	194	ND		
KP132	*uvrB*	+	120	240	2.3	2.0
KP188	*uvrB*	*4*	272	ND		
ES1597	*uvrD*	+	363	719	1.1	2.0
GM2164	*uvrD*	*13*	385	ND		
GW1040	*dinD*	+	13	130	1.8–2.6	10
GM1829	*dinD*	*4*	24–32	NA		
KP212	*dinF*	+	33	436	3.3	13
KP211	*dinF*	*4*	110	ND		

Cultures were grown and *β*-galactosidase activities were assayed as described in Materials and methods. The data for *dinD* are from Craig et al. (1984)

ND = not done; NA = not applicable

nation, we propose that this is one of the additional required $recA^+$ functions for dam^- viability. Supporting a role for recombination in dam^- strains is that the *mutS* and *mutL* alleles which suppress dam^- $recA^-$ lethality display a hyper-recombination phenotype (S. Feinstein and K.B. Low, personal communication). The viability of a *recA430* dam^- strain (see Table 2) suggests that the limited ability of RecA430 protein to inactivate LexA repressor is sufficient to fulfill this requirement and that the mutagenic function of RecA protein is not necessary for *dam* mutant viability. SOS mutagenesis is also demonstrated not to be required in dam^- strains because a *dam umuC* double mutant is viable (see Table 2). Increased expression of $recA^+$ is not necessary since a down-mutation in the *recA* promoter (*recA453*, formerly *tif-1 zab-53*; Castellazzi et al. 1972; Casaregola et al. 1982) is not lethal in dam^- strains (see Table 2). In addition, derepression of $recA^+$ is not adequate for *dam* mutant viability since for dam^- *lexA*(Ind$^-$) *recA*oc (operator constitutive) is an inviable combination (see Table 2). Further studies with *recA* mutants should identify which activities are necessary in dam^- backgrounds. In summary, strains lacking DNA adenine methylase activity require $recA^+$ function for LexA repressor inactivation and probably recombination.

Expression of SOS functions is increased in dam⁻ strains

If our interpretation of the *dam* interaction with the various *lexA* and *recA* alleles is correct, then expression of some or all of the SOS genes should be increased in dam^- strains. To test this hypothesis, *β*-galactosidase activity from *lacZ* operon fusions to *recA, lexA, sulA, uvrA, uvrB, uvrD, dinD* and *dinF* was measured in dam^+ or dam^- isogenic strains. In all cases, except for *uvrD*, *β*-galactosidase activity was increased in a dam^- background compared to a dam^+ background (see Table 5). We believe that this partial derepression is, at least in part, compensating for some as yet unknown lethal consequence of failure to methylate -GATC- sequences. It should be noted that the increased expression of these genes in dam^- strains is probably not due to stimulation of transcription in the absence of methylation. No *dam* methylation sites are part of or overlap the −35 or −10 regions of the *recA, lexA, uvrA* or *uvrB* genes (Hawley and McClure 1983). A *dam* methylation site does occur within the −35 region of the *sulA* promoter (Cole 1983) and this leads to increased activity of the promoter in the absence of *dam* methylation (S. Gottesman, personal communication). The six fold increase in *sulA* activity in *dam* mutants (see Table 5) probably reflects the sum of the combined effects of partial SOS induction and demethylation of the *dam* methylation site in the promoter. This reasoning is based on the observation that the *trpR* promoter, which also contains a *dam* methylation site within the −35 region, is only 2–3 times more active in *dam* mutants (Marinus 1985).

ruv gene product is required for dam mutant viability

At least one gene other than *recA* under LexA repressor control must be expressed since LexA repressor inactivation, but not RecA derepression, is required for *dam* mutant viability. In an effort to identify the other SOS gene(s) required for viability, we attempted to construct mutants of all known operons in the SOS regulon (except *recN*) in combination with a *dam* mutation (Table 2). All were viable except dam^- ruv^-.

Role of SOS functions in dam⁻ strains

In dam^- strains mismatch repair does not discriminate adequately between parental and daughter DNA strands, since there is no methylated DNA strand to serve as a preferential template for repair (Lu et al. 1983; Pukkila et al. 1983). *dam lig* mutants have an increased occurrence of single-stranded breaks (Marinus and Morris 1975) that may result from random mismatch correction. These same damage sites may function first as inducing signals for the SOS response and subsequently as substrates for SOS-related DNA repair or recombination. $recA^+$ and ruv^+ are both essential functions in dam^- strains and both are components of the SOS-inducible RecF pathway of recombination (Armengod 1982; Shurvington and Lloyd 1982; Lovett and Clark 1983; Lloyd et al. 1984). Three other genes are known to be involved in the RecF pathway of recombination, *recF, recJ*, and *recN* (Horii and Clark 1973; Lloyd et al. 1983). Like *recA* and *ruv, recN* is under control of *lexA* (Lloyd et al. 1983); *recF* is not and *recJ* remains to be tested (Lovett and Clark 1984). $recN^+$ has been shown to be required for repair of double-strand breaks (Picksley et al. 1984), which may persistently occur in *dam* mutants. We suggest that this recombination pathway is necessary, in addition to or instead of the RecBC pathway of recombination, to compensate for the chromosomal damage incurred as a result of being DNA adenine methylase deficient. Further evidence to support this hypothesis is that a dam^- $recJ^-$ strain could not be constructed (see Table 2). The viability of a *recF dam* double mutant (see Table 2) can be explained by the assumption that $recF^-$ does not provide a complete block to the necessary RecF recombination pathway functions as do $recA^-$, ruv^- and $recJ^-$ (Horii and Clark 1973; Ganesan and Seawell 1975; Roberts and Roberts 1975; Ar-

mengod and Blanco 1978; McPartland et al. 1980; Armengod 1982; Lloyd et al. 1984). *recF*$^+$ plays a role in the activation of the *recA*$^+$ proteolysis function following certain inducing treatments (McPartland et al. 1980; Karu and Belk 1982; Salles and Paoletti 1983). *recBC*$^+$ has also been implicated in the activation of RecA protein following inducing treatments different from those that require *recF*$^+$ (Oishi et al. 1978; Irbe and Oishi 1980; McPartland et al. 1980; Smith 1983); activation of RecA protein may proceed via this route in *dam* mutants and as such *recF*$^+$ may be dispensable. *recA, recB* and *recC* are all constituents of the RecBC pathway of recombination (Clark 1973). *recB* or *recC dam* double mutants are inviable (Marinus and Morris 1974). This may indicate a requirement for the RecBC recombination pathway and/or *recBC*$^+$ may be required to activate RecA protein. *sbcA* and *sbcB* mutations suppress the lethality of *recBC*$^-$ *dam*$^-$ strains as well as allowing expression of the RecE (*rac* cryptic prophage) or RecF pathways of recombination, respectively (Barbour et al. 1970; Templin et al. 1972; McGraw and Marinus 1980). In *dam*$^-$ *recBC*$^-$ *sbcA*$^-$ or *sbcB*$^-$ backgrounds, the RecBC recombination pathway is inactivated, suggesting that either the RecF or the RecE recombination pathway is sufficient to prevent lethality of a *dam* mutant. This further implies that *recBC*$^+$ may be required to activate RecA protein thus inducing the RecF pathway of recombination. Experiments are currently underway to determine the recombinational repertoire required for *dam*$^-$ viability.

Two secondary phenotypes of *dam* mutants, increased spontaneous prophage induction and the hyper-recombination phenotype, may be associated with the increased expression of SOS functions. In wild-type cells, derepression of the SOS response following inducing treatments results in the induction of several coliphages and in an enhanced capacity for DNA repair and recombination (Witkin 1976; Little and Mount 1982; Walker 1984 and references therein).

Subinduction of the SOS response in dam$^-$ *strains*

Although expression of several SOS operons is increased in *dam*$^-$ strains compared to *dam*$^+$ strains, none of the fusions examined are fully induced, except *lexA* and *uvrB* (see Table 5). *E. coli* has, in effect, adjusted the basal level of SOS expression to compensate for the condition of the intracellular environment without total derepression of functions within the regulon. We believe this to be a demonstration of a so-called subinduced state (Bailone et al. 1979; Little 1983; Walker 1984), a condition in which expression of the SOS responses is increased, but not maximally.

sin mutants

Mutations which *s*uppress the *in*viability (*sin*) of *dam*$^-$ *recA*$^-$ strains have been described (McGraw and Marinus 1980). Unlike *mutL* and *mutS* suppressor mutations, *sin*$^-$ strains are proficient at repairing base pair mismatches (P. Pukkila, unpublished data). It is possible that the *sin* gene product(s) is (are) involved either directly or indirectly in regulating or is an integral part of the SOS regulon. It has been difficult to analyse *sin*$^-$ strains since there is at present no obvious phenotype except Sin.

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