JOURNAL OF BACTERIOLOGY, Nov. 1993, p. 7505–7508
0021-9193/93/227505-040$02.00/0

Vol. 175, No. 22

Analysis of the Genetic Requirements for Viability of *Escherichia coli* K-12 DNA Adenine Methylase (*dam*) Mutants

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RecBCD protein, necessary for *Escherichia coli dam* mutant viability, is directly required for DNA repair. Recombination genes *recF*$^+$, *recN*$^+$, *recO*$^+$, and *recQ*$^+$ are not essential for *dam* mutant viability; they are required for *recBC sbcBC dam* mutant survival. *mutH*, *mutL*, or *mutS* mutations do not suppress subinduction of SOS genes in *dam* mutants.

Escherichia coli DNA adenine methylase (*dam*) mutants fail to methylate -GATC- sequences in double-stranded DNA (2, 18, 20, 22). As a consequence, methyl-directed mismatch repair fails to recognize the normally methylated parental strand that serves as a template for mismatch correction following passage of the DNA polymerase complex during DNA replication. These mutants display a variety of phenotypes including increased sensitivity to DNA-damaging agents (such as 2-aminopurine), hyperrecombination, increased spontaneous mutability, increased spontaneous induction of prophages, increased precise excision of certain transposons, and subinduction of genes of the SOS regulon (1, 6, 9, 10, 16, 21–24, 29, 37).

The SOS response is induced following treatments that damage DNA or inhibit DNA replication. RecA protein is activated by these treatments, and this activated form facilitates the cleavage of LexA repressor, a negative regulator of approximately 20 unlinked operons involved in DNA repair and mutagenesis (35). *dam* mutants require increased expression of the SOS regulon for viability (29). *recA dam* and *lexA*(Ind$^-$) *dam* double mutants are inviable, demonstrating that increased expression of at least some SOS genes is necessary for mutant survival (22, 23). Specifically, *recA*$^+$ and *ruv*$^+$ are necessary; the other tested SOS genes are expendable (29). Increased synthesis of RecA protein is not necessary, but the RecA gene product is required in a role other than just for LexA repressor inactivation. Apparently, basal levels of Ruv protein(s) are not adequate, and induction of the SOS genes results in sufficient production of gene product(s) from this locus. In addition, RecB, RecC, and RecJ gene products are required for viability (22, 29). These observations taken together suggest that some form of inducible recombination repair is necessary to compensate for being *dam*.

In this study, we tried to further elucidate the role of *recBCD*$^+$ in this process: whether it was required only to process or produce an SOS induction signal or whether it served a more direct role in the actual repair of constitutive DNA damage in *dam* hosts. P1 grown on a *recB* host was used to transduce *lexA51*(Def) (KP433), *lexA51*(Def) *dam* (KP432), *lexA51*(Def) *recA730*(Prt^c) (KP453), and *lexA51*(Def) *recA730* (Prt^c) *dam* (KP455) strains (Table 1) (26). *recB* transductants were recovered only from *dam*$^+$ recipients (KP433 and KP453). Table 2 shows that, even in a *dam* background where LexA repressor is permanently inactivated and RecA protein is constitutively activated for all its activities [*dam lexA*(Def) *recA*(Prt^c)], the quadruple mutant with *recB* could not be recovered. RecBCD protein has been implicated in DNA signal generation for activation of RecA protein and subsequent induction of the SOS response but should be dispensable in a *lexA*(Def) *recA*(Prt^c) host. Since *recB*$^+$ function is essential in this mutant, we infer that RecBCD proteins are required directly for repair.

We also wished to identify which, if any, of the remaining known recombination (*rec*) genes from the RecBC and RecF pathways of recombination in *E. coli* are necessary for *dam* viability. P1 grown on a *dam* host was used to transduce *recN*, *recO*, and *recQ* recipient strains (Table 1). Table 2 shows that *recF dam*, *recN dam*, *recO dam*, and *recQ dam* mutants were recovered; thus, *recF*$^+$, *recN*$^+$, *recO*$^+$, and *recQ*$^+$ are dispensable in a *dam* strain. *recG* and *recR* mutations were not tested (13, 17, 33). We were surprised by the *recN* result, since it is known to be an SOS gene and to be a component in double-strand break repair (30). Double-strand breaks have been implicated as the type of damage found in *dam* strains responsible for inviability in certain genetic backgrounds (36).

In *recBC sbcBC* strains, the RecBC pathway of recombination is inactivated but the RecF pathway of recombination is functional (3, 34). Exonuclease I (Exo I) activity, which is encoded by the *sbcB* gene, is eliminated, and the SOS genes are turned on, as evidenced by increased expression of *recA* and *sulA lacZ*$^+$ operon fusions (Table 3). *dam recBC* mutants are viable if they harbor *sbcBC* mutations (25). Table 2 shows that *dam recBC sbcBC* strains require all the known genes of the RecF pathway, except *recG* and *recR*, which were not tested. P1 grown on *recF*, *recN*, *recO*, or *recQ* hosts was used to transduce these mutations into a *recB recC sbcBC* strain (JC9387, a gift from A. J. Clark; Table 1). Transductants with the appropriate drug resistance and the expected phenotype associated with each *rec* mutant were obtained. The transduction frequency in a *dam* derivative of this strain (KP418) dropped 2 to 3 log units, to 0. The converse crosses were also attempted; i.e., P1 grown on a *dam* strain was used to transduce the *recB recC sbcBC* strain (JC9387) and derivatives of this strain containing *recF* (KP423), *recN* (KP421), *recO* (KP422), or *recQ* (KP368) mutations (Table 1). Transductants were recovered only from the *recB recC sbcBC* recipient (Table 2). Thus, *recB recC sbcBC dam* mutants require function of all tested genes in the RecF pathway of recombination.

Our data demonstrate that *dam* mutant viability depends upon the function of at least one recombination pathway for repair of DNA damage constitutive in these mutants. We

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TABLE 1. *E. coli* K-12 strains

Strain	Relevant genotype[a]	Source or reference
DE272	*srlC300*::Tn*10 recA730 lexA51*(Def) *sulA211* Δ(*lac-gpt*)*5 rpsL31 thi-1* (*xyl-5*)? (*ara-14*)? *mtl-1* (*tsx-33*)? *ilv*(ts) λ$^-$ *supD43*	7
DM2568	*lexA51*(Def) *sulA211 srlC300*::Tn*10 thi-1* (*ara-14*)? (*xyl-5*)? *mtl-1 rpsL31* (*tsx-33*)? *ilv*(ts) *supD43* Δ(*lac-gpt*)*5*	7
JC9387	*rpsL31 supE44 thr-1 leuB6 proA2 his-4 thi-1 argE3 galK2 lacY1 ara-14 xyl-5 mtl-1 tsx-33 kdgK51* λ$^-$ F$^-$	A. J. Clark
KD1996	*recQ61*::Tn*3*	28
KP317	As SP256 but *dam13*::Tn*9*	This study[b]
KP319	As SP194 but *dam13*::Tn*9*	This study[b]
KP363	As KD1996 but *dam13*::Tn*9 cysG*::Tn*5*	This study[c]
KP368	As JC9387 but *recQ61*::Tn*3*	This study[d]
KP370	As RDK1541 but *dam13*::Tn*9*	This study[c]
KP418	As JC9387 but *dam13*::Tn*9*	This study[b]
KP421	As JC9387 but *tyr16*::Tn*10 recN262*	This study[e]
KP422	As JC9387 but *recO1504*::Tn*5*	This study[f]
KP423	As JC9387 but *recF232*::Tn*3*	This study[g]
KP432	As DM2568 but *dam13*::Tn*9 cysG*::Tn*5 srl* Tc^s	This study[c]
KP433	As DM2568 but *srl* Tc^s	This study
KP449	As KP433 but *recB268*::Tn*10*	This study[h]
KP453	As DE272 but *srl* Tc^s	This study
KP455	As DE272 but *dam13*::Tn*9 cysG*::Tn*5 srl* Tc^s	This study[c]
KP498	*srl sulA211 lac* Δ(U169) *thr-1 leu-6 his-4 argE3 ilv*(ts) *galK2 rpsL31 supE44* λcI(Ind$^-$) *recA*::*lac cysG*::Tn*5*	This study[i]
KP522	*rpsL31 supE44 thr-1 leuB6 proA2 his-4 thi-1 argE3 galK2 lacY1 ara-14 xyl-5 mtl-1 tsx-33 kdgK51* F$^-$ *lacZ950*::Tn*10* λcI(Ind$^-$) *recA*::*lac*	This study[i]
KP523	As KP522 but *recB21 recC22 sbcBC15*	This study
KP524	As KP522 but λcI(Ind$^-$) *sulA*::*lacZ*	This study[j]
KP525	As KP524 but *recB21 recC22 sbcBC15*	This study
NO22	HfrH Δ*lac relA thi zja505*::Tn*10 sulA*::Mud(Ap *lac*) (Mu$^+$) λ$^-$	29
RDK1541	*recO1504*::Tn*5*	11
SP194	*recN259*::Mud(Ap *lac*)	15
SP256	*recN262*	14

[a] Tc^s, tetracycline sensitive.
[b] *dam13*::Tn*9* was transduced from P1 grown on KP301.
[c] *dam13*::Tn*9* and/or *cysG*::Tn*5* was transduced from P1 grown on KP315.
[d] *recQ61*::Tn*3* was transduced from P1 grown on KP409.
[e] *tyr16*::Tn*10 recN262* was transduced from P1 grown on KP408.
[f] *recO1504*::Tn*5* was transduced from P1 grown on KP410.
[g] *recF232*::Tn*3* was transduced from P1 grown on KP412.
[h] *recB268*::Tn*10* was transduced from P1 grown on KP425.
[i] λcI(Ind$^-$) *recA*::*lac* from reference 29.
[j] λcI(Ind$^-$) *sulA*::*lacZ* from reference 12.

TABLE 2. DNA recombination and repair genes required for *dam* mutant viability[a]

Genetic background	
Inviable	Viable
dam recA (22)	*dam recF* (29)
dam recB (22)	*dam recN*
dam recC (22)	*dam recO*
dam recJ (29)	*dam recQ*
dam ruv (29)	
dam lexA51(Def) *recB*	
dam lexA51(Def) *recA730 recB*	
dam recBC sbcBC recF	*dam recBC sbcBC* (25)
dam recBC sbcBC recN	
dam recBC sbcBC recO	
dam recBC sbcBC recQ	

[a] P1 *vir* phage transductions were performed by a standard genetic method (26).

propose that DNA double-strand breaks associated with non-directed mismatch repair processes in *dam* mutants require repair via a RecBC-dependent route involving, at a minimum, the products of *recA*$^+$, *recB*$^+$, *recC*$^+$, *recJ*$^+$, and *ruv*$^+$. In genetic backgrounds where the RecBC pathway is inactivated, repair occurs through the SOS-inducible RecF pathway of recombination, requiring functional *recA*$^+$, *recF*$^+$, *recJ*$^+$, *recN*$^+$, *recO*$^+$, and *recQ*$^+$ genes.

The inviability of *dam recBCD* mutants may be the result of the following scenario. When exonuclease I (*sbcB*$^+$ gene product) is functional, *recBC*$^+$ is required to act on Exo I-processed substrates. When *recBC*$^+$ is inactivated, Exo I must also be knocked out in order for *dam*-generated damage to be preserved in a state that can be recognized and repaired via the RecF pathway. *sbcBC* mutations may serve a dual role; they knock out Exo I activity while concomitantly turning on the SOS-inducible RecF pathway of recombination.

mutH$^+$, *-L*$^+$, and *-S*$^+$ encode genes involved in methyl-directed mismatch repair (4, 27, 31). Mutations in any one of these genes in a *dam* host reverse most of the aforementioned phenotypes associated with DNA adenine methylase mutations (8, 25). We wished to test whether *mut* mutations reverse

TABLE 3. Constitutive expression of SOS genes by *E. coli recBC sbcBC* mutants[a]

Strain	Fusion	Miller units
KP522 (wild type)	*recA*	724
KP523 (mutant)	*recA*	2,233
KP524 (wild type)	*sulA*	84
KP525 (mutant)	*sulA*	618

[a] Cultures were grown and β-galactosidase activities were assayed as described by Peterson et al. (29).

the increased expression of SOS genes. In the absence of a methylated DNA template to direct mismatch repair, MutHLS proteins may generate random breaks in the DNA. These breaks may serve to induce the SOS response. We predict, then, that *mut* mutations should prevent the formation of these signal lesions and the concomitant increase in expression of the SOS genes. We used *lacZ*$^+$ operon fusions to *sulA* and *recA* promoters to measure expression of the SOS response in both *dam*$^+$ and *dam* backgrounds in the presence or absence of *mut* alleles (Table 4). We used the *recA* fusion in addition to *sulA* because there is a GATC site in the *sulA*$^+$ promoter that makes expression of this gene partially dependent on its state of methylation (5, 19). While the *mut* alleles alleviated 2-aminopurine sensitivity of the *dam* strains (data not shown), they did not decrease expression of the SOS genes (Table 4).

Other work has demonstrated that *mut* mutations prevent formation of DNA lesions thought to be potentially lethal in *dam* cells (double-strand breaks) (36). Therefore, we assume (although we have not investigated it) that the genes required for *dam* viability should be dispensable in *mut* backgrounds.

TABLE 4. Lack of suppression by *mut* alleles of increased expression of the SOS regulon in *E. coli dam* strains[a]

Strain	Fusion	Allele		β-Gal activity (Miller units)
		dam	*mut*	
KP499	*sulA*	+		90
KP507	*sulA*	+	*mutL*	96
KP506	*sulA*	+	*mutS*	92
KP516	*sulA*	+	*mutH*	92
KP483	*sulA*	−		603
KP490	*sulA*	−	*mutL*	547
KP489	*sulA*	−	*mutS*	749
KP517	*sulA*	−	*mutH*	557
KP498	*recA*	+		729
KP505	*recA*	+	*mutL*	742
KP504	*recA*	+	*mutS*	774
KP518	*recA*	+	*mutH*	859
KP484	*recA*	−		4,313
KP492	*recA*	−	*mutL*	5,105
KP491	*recA*	−	*mutS*	5,279

[a] Cultures were grown and β-galactosidase (β-Gal) activities were assayed as described by Peterson et al. (29). KP499, KP507, KP506, KP516, KP483, KP490, KP489, and KP517 are all Tc^s derivatives of NO22 (Table 1) (29). KP483, KP490, KP489, and KP517 were made *dam-4 cysG*::Tn*5* by transduction from P1 grown on GM1737 (1). KP483 was made *cysG*$^+$ Km^s (kanamycin sensitive) by selection on M9 plates lacking cysteine prior to transduction with P1 grown on GW3814 *mutH*::Tn*5* (gift of G. C. Walker) to produce KP517. Strains used as donors of *mut* alleles in P1 transductions were ES1484 *mutL218*::Tn*10* (32), ES1481 *mutS215*::Tn*10* (32), and GW3814 *mutH*::Tn*5*. KP505, KP504, KP518, KP484, KP492, and KP491 are isogenic with KP498 (Table 1). KP484, KP492, and KP491 are, in addition, *dam-4*. KP518 was derived by transduction of KP497 *srl*::Tn*10* *cysG*$^+$ Km^s with P1 grown on GW3814 *mutH*::Tn*5*; otherwise it is isogenic with the other strains of this group. +, wild type; −, mutant.

We believe that, in addition to the potentially lethal DNA damage constitutively produced in *mut*$^+$ *dam* strains, another, presumably nonlethal, DNA substrate that serves as an SOS inducing signal is produced. This does not preclude the former as an inducer as well. We do not know the nature of this signal; perhaps undermethylated DNA itself functions to turn on the SOS response.

Thanks to A. J. Clark, D. G. Ennis, R. A. Fishel, J. W. Little, R. G. Lloyd, M. G. Marinus, H. Nakayama, N. Ossanna, E. C. Siegel, G. C. Walker, and K. F. Wertman for bacterial and phage strains. Thanks to D. G. Ennis, M. G. Marinus, P. A. Navas, and P. Poorkaj for critically reading the manuscript.

This work was supported by Public Health Service grant GM24496, awarded to D.W.M.

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