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DNA Adenine Methylase Mutants of *Salmonella typhimurium* and a Novel Dam-Regulated Locus

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ABSTRACT

Mutants of *Salmonella typhimurium* lacking DNA adenine methylase were isolated; they include insertion and deletion alleles. The *dam* locus maps at 75 min between *cysG* and *aroB*, similar to the *Escherichia coli dam* gene. Dam⁻ mutants of *S. typhimurium* resemble those of *E. coli* in the following phenotypes: (1) increased spontaneous mutations, (2) moderate SOS induction, (3) enhancement of duplication segregation, (4) inviability of *dam recA* and *dam recB* mutants, and (5) suppression of the inviability of the *dam recA* and *dam recB* combinations by mutations that eliminate mismatch repair. However, differences between *S. typhimurium* and *E. coli dam* mutants are also found: (1) *S. typhimurium dam* mutants do not show increased UV sensitivity, suggesting that methyl-directed mismatch repair does not participate in the repair of UV-induced DNA damage in Salmonella. (2) *S. typhimurium dam recJ* mutants are viable, suggesting that the Salmonella RecJ function does not participate in the repair of DNA strand breaks formed in the absence of Dam methylation. We also describe a genetic screen for detecting novel genes regulated by Dam methylation and a locus repressed by Dam methylation in the *S. typhimurium* virulence (or "cryptic") plasmid.

THE biological functions of DNA adenine methylation have been widely investigated in *Escherichia coli* and its phages (reviewed by HATTMAN 1981; MARINUS 1984, 1987a,b; MESSER and NOYER-WEIDNER 1988; BARRAS and MARINUS 1989; NOYER-WEIDNER and TRAUTNER 1993). The DNA of *E. coli* contains ~1.5 mol of 6-methyl-adenine per 100 mol of adenine (HATTMAN 1981; MARINUS 1984). Formation of 6-methyl-adenine results from postreplicative modification of adenine residues in 5′-GATC-3′ sites; methylation occurs in both strands of the palindromic target (LACKS and GREENBERG 1977). The methylation reaction is catalyzed by the enzyme DNA adenine methyltransferase, often called "Dam methylase" (HERMAN and MODRICH 1982). The *dam* gene is located at 74 min on the *E. coli* genetic map and is cotransducible with *cysG* (MARINUS 1973; BACHMANN 1990). The *dam* gene is part of an operon containing *aroK, aroB, trpS* and two additional genes involved in carbohydrate metabolism (LØBNER-OLESEN *et al.* 1992; LYNGSTADAAS *et al.* 1995).

In *E. coli* and its phages Mu and P1, many roles of Dam methylation have been identified. Upon chromosome replication, the existence of hemimethylated 5′-GATC-3′ sites directs mismatch repair toward the newly synthesized, unmethylated strand (LU *et al.* 1983; PUKKILA *et al.* 1983; RADMAN and WAGNER 1986; MODRICH 1987). Dam methylation also controls the initiation of chromosome replication (MESSER *et al.* 1985; SMITH *et al.* 1985; BOYE and LØBNER-OLESEN 1990; CAMPBELL and KLECKNER 1990; ABELES *et al.* 1993), segregation of the daughter chromosome molecules (OGDEN *et al.* 1988; HERRICK *et al.* 1994), regulation of plasmid replication (RUSSEL and ZINDER 1987; GAMMIE and CROSA 1991), and the activity of certain host and phage genes (HATTMAN 1982; MARINUS 1985; ROBERTS *et al.* 1985; BRAUN and WRIGHT 1986; KÜCHERER *et al.* 1986; BLYN *et al.* 1990; CAMPBELL and KLECKNER 1990; BRAATEN *et al.* 1994). In addition, Dam methylation is involved in the initiation of P1 DNA replication (ABELES *et al.* 1993), packaging of bacteriophage P1 DNA into virions (STERNBERG and COULBY 1990) and also affects functions associated with bacterial retroposons (HSU *et al.* 1990). This variety of processes affected by Dam methylation confirms earlier expectations on its relevance as a physiological signal (HATTMAN 1981; MARINUS 1987a,b).

A *Salmonella typhimurium* point mutant lacking DNA adenine methylase was described a decade ago (RICHTIE *et al.* 1986). Given the pleiotropy of *dam* mutations of *E. coli* and the intricacies found in their manipulation (MCGRAW and MARINUS 1980; PETERSON *et al.* 1985; PETERSON and MOUNT 1993), we judged that the study of Dam methylation in Salmonella might become easier if insertion and deletion alleles were available. Both types of alleles are described in this paper. In addition, a detailed characterization of *S. typhimurium* Dam⁻ mutants shows that they share with those of *E. coli* many defects in recombination and DNA repair; however, differences are also found. The latter are intriguing and

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potentially insightful, like other examples of divergence between Escherichia and Salmonella (RILEY and SANDERSON 1990).

The last part of this study describes a genetic screening for the detection of genes regulated by Dam methylation and the finding of a locus strongly repressed by Dam methylation, located in the *S. typhimurium* virulence plasmid.

MATERIALS AND METHODS

Bacterial strains, bacteriophages and strain construction: The *S. typhimurium* strains used in this study are listed in Table 1. Transductional crosses using phage P22 HT 105/1 *int201* (SCHMIEGER 1972; G. ROBERTS, unpublished data) were used for strain construction operations involving chromosomal markers; the transducing phage will be henceforth referred as "P22 HT". Phage sensitivity was tested by cross-streaking with the clear-plaque mutant P22 H5. To obtain phage-free isolates, transductants were purified by streaking on "green" plates. Strains SV3019–SV3030 arose from matings between the *E. coli* donors CC101–CC106 (CUPPLES and MILLER 1989) and either of the *S. typhimurium* recipients SV3016 or SV3017. In these crosses, F-prime transfer was scored on E plates, selecting Pro^+ Tet^r or Pro^+ Cam^r transconjugants; growth in the absence of proline selected for F-prime transfer, while the presence of the antibiotic counterselected the *E. coli* donors. Strain UA1570, obtained from Xavier Garriga (Dep. Microbiologia, Universitat Autònoma de Barcelona, Bellaterra, Spain), carries a Tn*5* chromosomal insertion that has not been mapped; we have named this insertion *zzz-6305*, following the nomenclature of CHUMLEY *et al.* (1979) and using a *z*– allele number assigned to our laboratory by the Salmonella Genetic Stock Center (SGSC), University of Calgary, Alberta, Canada. The nature and the origin of this Tn*5* insertion are described in the following section. Using the same nomenclature system, a Mu*dJ* insertion in an unknown locus of the *S. typhimurium* virulence plasmid has been named *zzv-6306*::Mu*dJ*.

Plasmids and transposons: F'128 *pro*$^+$ *lac*$^+$ *zzf-1836*::Tn*10d*Cam is an *E. coli* episome carrying a defective Tn*10* element that confers cloramphenicol resistance (ELLIOTT and ROTH 1985). pNK972 (Amp^r) is a pBR332 derivative carrying the IS*10* transposase gene under the control of a *tac* promoter (BENDER and KLECKNER 1986). The *E. coli* episome F'128 *pro*$^+$ *lac*$^+$ *zzf-1831*::Tn*10d*Tet and the *S. typhimurium* episome F'152 *nad*$^+$ *zzf-1833*::*Tn10d*Kan were both obtained from J. R. ROTH, Department of Biology, University of Utah, Salt Lake City, Utah. Plasmid pTP166, obtained from M. G. MARINUS (Department of Pharmacology, University of Massachusetts, Worcester, Massachusetts), is a pBR322 derivative carrying the *E. coli dam* gene under the control of a *tac* promoter (MARINUS *et al.* 1984). Repression of the *tac* promoter was achieved by using the episome F' *pro*$^+$ *lacI*^{q} *L8Z*::Tn*10Δ4-HH104*, obtained from J. R. ROTH. Plasmid pGE108 (Kan^r) is a ColE1 derivative carrying a *cea*::*lacZ* fusion (SALLES *et al.* 1987). Plasmid pSE143 (Kan^r) is a pSC101 derivative carrying a *umuDC*::*lacZ* fusion (ELLEDGE and WALKER 1983). pIZ53 is a pUC19 derivative carrying the internal *Hin*dIII fragment of Tn*5*; this fragment includes the kanamycin-resistance gene (MALDONADO *et al.* 1992). Mu*d*I is the specialized transducing phage Mu*d*I (Amp Lac *c*ts62) originally constructed by CASADABAN and COHEN (1979). Mu*d*I-8 is a transposition-defective derivative of Mu*d*I (HUGHES and ROTH 1984). Mu*d*I1734[Kan-Lac] (CASTILHO *et al.* 1984) is a transposition-deficient Mu derivative that generates operon fusions upon insertion; the element has been renamed Mu*dJ* (HUGHES and ROTH 1988). Mu*dQ* is a "locked-in" P22-Mu hybrid conferring chloramphenicol resistance (YOUDERIAN *et al.* 1988; BENSON and GOLDMAN 1992). The insertion allele *zzz-6305*::Tn*5*[lexA::*lacZ*] carries a defective Tn*5* derivative bearing a transcriptional fusion between the *S. typhimurium lexA* promoter/operator region and the *E. coli lacZ* gene (GARRIGA 1992).

Media and growth conditions: The E medium of VOGEL and BONNER (1956) was used as the standard minimal medium. NCE is E medium without citrate. Carbon sources were either 0.2% glucose or 1% lactose. The rich medium was nutrient broth (8 g/l, Difco) with added NaCl (5 g/l). MacConkey agar base was from Difco. Solid media contained Difco agar at 1.5% final concentration. Auxotrophic requirements and antibiotics were used at the final concentrations described by MALOY (1990). Green plates were prepared according to CHAN *et al.* (1972), except that methyl blue (Sigma) substituted for aniline blue. For the selection of tetracycline-sensitive derivatives of Tet^r strains, we used the medium of BOCHNER *et al.* (1980) as modified by MALOY and NUNN (1981).

Transposon substitutions: A lysate grown on strain TT10425 was used for transposon replacement at the *dam* locus. This strain carries an F prime containing Tn*10d*Kan (see the strain list). The lysate was irradiated with UV light, using a 15 W Sylvania lamp at a distance of 30 cm for 30 sec; irradiation of the phage suspensions can be expected to increase recombination in the transductants (RUPP *et al.* 1971). Transductions selecting the incoming marker (Kan^r) were carried out. Kan^r transductants were then scored for loss of the resident marker (Tet^r). This procedure allowed us to obtain allele variants tagged with different antibiotic resistance markers (*e.g.*, Tet^r and Kan^r). Replacement of a *lacZ* allele with the *lacZ477*::Tn*10-d*Tet insertion was achieved using a lysate grown on strain TT16716. The lysate was UV irradiated as above; Tet^r transductants were selected on lactose indicator plates supplemented with tetracycline.

Measurement of spontaneous mutation rates using *lac* alleles: Aliquots containing 10^6 cells were added to six tubes of NCE liquid medium containing 0.2% glucose. The cultures were incubated at 37° until saturation ($\sim 10^9$ cells/ml). Cells were then spread on NCE-lactose plates (10^8 cells per plate, four plates per culture). Revertants were scored after 48-hr incubation at 37°. Viable cell counts were carried out on NB plates.

Mutagenesis with Tn*10d*Cam and isolation of insertions linked to the *dam* locus: We used the "nonhomologous transduction" procedure (ELLIOTT and ROTH 1985), with modifications described elsewhere (FLORES and CASADESÚS 1995). A lysate grown on strain SV2056 was used to transduce strain SV3002, selecting Cam^r Amp^r transductants. Transducing mixtures were made on NB plates and preincubated for 4–6 hr at 37° before replica-printing to NB supplemented with chloramphenicol and ampicillin. Cam^r Amp^r transductants were replica-printed several (more than three) times to NB-Tet plates containing EGTA 10 mM; the latter was added to prevent reinfection (thus allowing the isolation of phage-free derivatives). Pools of 1000–2000 Cam^r Tet^r colonies were made and lysed with phage P22 HT. The pools were then used to transduce strain LT2; to detect cotransduction of the Tn*10d*Cam and Tn*10d*Tet elements, Tet^r transductants were replica-printed to NB plates supplemented with chloramphenicol.

Transposition of Mu*dJ* and random formation of transcriptional *lac* fusions: A P22 HT lysate grown on strain TT10288 (HUGHES and ROTH 1988) was used to transduce TT1704, selecting kanamycin resistance. The recipient strain carried the nontransducible deletion Δ*his-9533*. Transductants were

TABLE 1

Bacterial strains

Strain	Genotype or phenotype	Reference[a]
JC608	*dam-201*::Tn*10d*Tet *rpsL1*	
JC609	*dam-202*::Tn*10d*Tet *rpsL1*	
JC610	*dam-203*::Tn*10d*Tet *rpsL1*	
SV80	Δ*his-3050*	
SV1223	*recB503*::Tn*10*	Garzon *et al.* (1996)
SV1234	*recF522*::Tn*5*	A. Garzon
SV1240	*zzz-6305*:Tn5 [*lexA*::*lacZ*]	A. Garzon
SV1244	*recA1 recB497*::Mu*d*J	Garzon *et al.* (1995)
SV2056	Δ*his-3050*/F′ 128 *lac*$^+$ *pro*$^+$ *zzf-1836*::Tn*10d*Cam	Flores and Casadesús (1995)
SV3000	*dam-201*::Tn*10d*Tet	
SV3001	*dam-201*::Tn*10d*Kan	
SV3002	*dam-201*::Tn*10d*Tet/pNK972	
SV3003	*zzv-6306*::Mu*d*J	
SV3004	DUP [*trp-2482* *Mud1-8* *hisD9953*]	
SV3005	DUP [*trp-2482* *Mud1-8* *hisD9953*] *dam-201*::Tn*10d*Tet	
SV3006	Δ*dam-204*	
SV3007	*dam-201*::Tn*10d*Tet *zzz-6305*:Tn*5* [*lexA*::*lacZ*]	
SV3008	Δ*dam-204 zzz-6305*:Tn5 [*lexA*::*lacZ*]	
SV3009	LT2/pTP166	
SV3010	DUP [*trp-2482* *Mud1-8* *hisD9953*] *dam*Δ*204*	
SV3011	Δ*dam-204 mutL111*::Tn*10*	
SV3012	Δ*dam-204 mutS121*::Tn*10*	
SV3013	Δ*dam-204 mutH101*::Tn5	
SV3014	Δ*dam-204*/pTP166	
SV3015	DUP [*trp-2482* *Mud1-8* *hisD9953*] *dam*Δ*204 mutH101*::Tn*5*	
SV3016	*proA692*::Mu*d*Q	
SV3017	*dam-201*::Tn*10d*Tet *proA692*::Mu*d*Q	
SV3019	*proA692*::Mu*d*Q/F′ *lacZ101 proAB*$^+$	
SV3020	*proA692*::Mu*d*Q/F′ *lacZ102 proAB*$^+$	
SV3021	*proA692*::Mu*d*Q/F′ *lacZ103 proAB*$^+$	
SV3022	*proA692*::Mu*d*Q/F′ *lacZ104 proAB*$^+$	
SV3023	*proA692*::Mu*d*Q/F′ *lacZ105 proAB*$^+$	
SV3024	*proA692*::Mu*d*Q/F′ *lacZ106 proAB*$^+$	
SV3025	*dam-201*::Tn*10d*Tet *proA692*::Mu*d*Q/F′ *lacZ101 proAB*$^+$	
SV3026	*dam-201*::Tn*10d*Tet *proA692*::Mu*d*Q/F′ *lacZ102 proAB*$^+$	
SV3027	*dam-201*::Tn*10d*Tet *proA692*::Mu*d*Q/F′ *lacZ103 proAB*$^+$	
SV3028	*dam-201*::Tn*10d*Tet *proA692*::Mu*d*Q/F′ *lacZ104 proAB*$^+$	
SV3029	*dam-201*::Tn*10d*Tet *proA692*::Mu*d*Q/F′ *lacZ105 proAB*$^+$	
SV3030	*dam-201*::Tn*10d*Tet *proA692*::Mu*d*Q/F′ *lacZ106 proAB*$^+$	
SV3031	DUP [*trp-2482* *Mud1-8* *hisD9953*] *mutH101*::Tn*5*	
SV3069	*zzv-6306*::Mu*d*J *dam-201*::Tn*10d*Tet	
SV3070	*zzv-6306*::Mu*d*J Δ*his-3050*	
SV3071	*zzv-6306*::Mu*d*J Δ*his-3050 dam-201*::Tn*10d*Tet	
SV3072	*zzv-6306*::Mu*d*J Δ*his-3050*	
SV3073	*zzv-6306*::Mu*d*J Δ*his-9533 dam-201*::Tn*10d*Tet	
SV3074	*zzv-6306*::Mu*d*J [*lacZ*::Tn*10d*Tet]	
TR5527	Δ*his-712*, cured of the virulence plasmid	J. R. Roth
TR5667	*cysG439 rpsL1*	J. R. Roth
TR5878	r(LT2)$^-$ m(LT2)$^-$ r(S)$^+$ *ilv-542 met A22 trpB2* Fels2$^-$ *fliA66 rpsL120 xyl-404 metE551 hspL56 hspS29*	SGSC[b]
TT1704	Δ*his-9533*	J. R. Roth
TT2742	*aroB542*::Tn*5*	SGSC[b]
TT9286	*proAB47 leuD798 argI537 ara-9 fol-1*/F′128 *proA*$^+$*B*$^+$ *argF*$^+$ *lacI9 Z*$^+$ *Y*$^+$ *A*$^+$	J. R. Roth
TT10425	*nadA56*/F′152 *nad*$^+$ *zzf-1833*::Tn*10d*Kan	J. R. Roth
TT10288	*hisD9953*::Mu*d*J *hisA944*::Mud1	Hughes and Roth (1988)
TT10838	*recA1*	J. R. Roth
TT11289	*recA1 srl-203*::Tn*10d*Cam	J. R. Roth
TT15278	*recJ504*::Mu*d*J	Mahan *et al.* (1992)
TT16716	*his-644 pro-621*/F′ *pro*$^+$ *lacZ477*::Tn*10d*Tet	SGSC[b]
UA1570	*zzz-6305*::Tn*5* [*lexA*::*lacZ*] Rif^r	X. Garriga

[a] Omitted for strains first described in this study.

[b] SGSC: Salmonella Genetic Stock Center, Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

selected on lactose indicator media (MacConkey-lactose or X-gal NB plates) supplemented with kanamycin. Rare Km^r Ap^r transductants generated by double-fragment transduction are easily identified because they are Kan^r Amp^r, while transductants carrying Mu*dJ* "hops" are Kan^r Amp^s (HUGHES and ROTH 1988). Kan^r transductants were made phage-free by replica-printing (more than two times) to plates containing 10 mM EGTA.

Transduction and cotransductional mapping: Preparation of phage P22 lysates and transductions were performed according to GARZON *et al.* (1995). The efficiency of transduction (EOT) of a given lysate is the ratio between the numbers of transductants obtained on a pair of isogenic strains. For cotransductional mapping, transductants were replica-printed to suitable plates to score transduction of unselected markers. Cotransduction frequencies are averages of more than four independent crosses, scoring at least 300 transductants from each cross. The relative order of markers was determined by three-factor crosses. Table 1 contains the original strains carrying markers cotransducible with the loci studied but not their derivatives constructed by adding one of the alleles to be mapped; such constructions were required to perform certain three-factor crosses.

UV survival assays: Overnight cultures made in NB were diluted 1/10 in the same medium. When the cultures reached an $O.D._{600}$ = 0.5, the cells were harvested and resuspended in E buffer (E medium without glucose). Five-milliliter aliquots were transferred to sterile, empty Petri dishes. Irradiation was achieved by opening the plates under a 15 W Sylvania UV lamp at a distance of 30 cm in the absence of daylight illumination. Cell suspensions were stirred during irradiation. After serial dilution in foil-covered tubes, irradiated cultures were plated on NB.

Analysis of duplication segregation: A single colony from a selective plate was used to inoculate 2 ml of nonselective NB broth, grown overnight, diluted and plated on nonselective NB agar. When colonies appeared, they were replica-printed to plates selective for cells carrying the duplication (NB supplemented with ampicillin). The percentage given refers to the fraction of colonies that lost the duplication.

Rapid mapping with Mu*d*-P22 prophages: We followed the procedure of BENSON and GOLDMAN (1992), using a collection of 67 "locked-in" Mu*d*-P22 prophages (BENSON and GOLDMAN 1992; FLORES and CASADESÚS 1995). This collection is not included in the strain list.

β-galactosidase assays: Levels of β-galactosidase were assayed as described by MILLER (1972), using the $CHCl_3$-sodium dodecyl sulfate permeabilization procedure.

Isolation and purification of genomic DNA: A pellet from a 5 ml culture grown in NB was resuspended in 1 ml TE-glucose (25 mM Tris-HCl, 10 mM EDTA, 50 mM glucose pH 8.0) and 0.1 ml of a mixture containing lysozyme, proteinase K and ribonuclease A (final concentrations: 5 μg/ml, 0.05 μg/ml, and 0.5 μg/ml, respectively). After 30 min of incubation at 37°, 0.1 ml SDS (20%) was added and incubation was continued for another 30 min. DNA was sheared by passing once through a syringe and extracted once with phenol, twice with phenol-chloroform and twice with chloroform. DNA was precipitated at −70° by adding 2.5 volumes of absolute ethanol and 1/10 (v:v) of 3 M sodium acetate. After centrifugation, the DNA pellet was resuspended in 0.2 ml TE buffer (Tris-HCl 10 mM, EDTA 1 mM pH 7.6).

Discrimination of the methylation status of adenine residues in genomic DNA: Genomic DNA preparations were digested with restriction enzymes *Sau*3AI, *Dpn*I and *Mbo*I (all from Boehringer Mannheim). All these enzymes recognize the sequence 5′-GATC-3′. The endonucleolytic activity of *Mbo*I is blocked by Dam methylation, while *Dpn*I only cuts methylated DNA; cutting by *Sau*3AI is irrespective of the methylation status.

Transformation of *S. typhimurium*: The transformable strain TR5878 was used as the recipient of plasmids; preparation of competent cells and transformation followed the procedures of LEDERBERG and COHEN (1974). Plasmids transformed into TR5878 were transferred to suitable recipients by transduction with P22 HT.

Extraction of the *S. typhimurium* virulence plasmid: One milliliter of an overnight culture in LB was centrifuged at 12,000 rpm for 2 min at 4°. The pellet was resuspended in 150 μl of E buffer; 300 μl of lysis solution were then added. After incubating at 65° for 1 hr, the lysate was chilled on ice and shaken for 10 min (until a white precipitate was formed). The preparation was then buffered by adding 150 μl of ice-cold 2 M Tris, shaken gently until it became transparent and centrifuged at 12,000 rpm for 20 min in the cold. The supernatant was transferred to a clean tube and mixed with one volume of nonsaturated phenol:chloroform:isoamyl alcohol (25:24:1). After two to three extraction cycles, DNA was precipitated with 3 M sodium acetate and absolute ethanol. The pellet was rinsed with 70% ethanol and resuspended in 10 μl of minimal TE. All preparations were treated with ribonuclease (0.1 mg/ml, final concentration) before storage at −20°.

DNA hybridization: DNA hybridization followed the procedures described by SAMBROOK *et al.* (1989). DNA was transferred to a nylon membrane using a vacuum blotting system (TransVac TE80, Hoeffer Scientific Instruments) and cross-linked by UV irradiation. The probes were labeled by random priming with chemiluminescent digoxigenin-dUTP (Boehringer Mannheim); hybridization bands were visualized on an X-ray film.

RESULTS

Isolation and characterization of Dam^- mutants of *S. typhimurium*: Tn*10d*Tet insertions in the *S. typhimurium dam* locus were identified following localized mutagenesis of the region known to include this gene in *E. coli.* In the latter, *dam* is located between *cysG* and *aroB* at min 74 (MARINUS 1973; BACHMANN 1990), a region that corresponds to min 75 in *S. typhimurium* (SANDERSON *et al.* 1995). In our search for Tn*10d*Tet insertions in *dam*, the *cysG* gene was used as the linked marker because mutations in the closer gene *aroB* often cause a leaky Dam^- phenotype (data not shown); a similar effect of *aroB* mutations has been described in *E. coli* (LØBNER-OLESEN *et al.* 1992). Regional mutagenesis with Tn*10d*Tet was achieved in two steps: (1) nine pools of Tn*10d*Tet insertions were generated in the wild-type strain LT2; each pool was made from 2500–3000 independent Tet^r colonies; (2) P22 HT phage grown on the pooled cells was used to transduce strain TR5667, selecting tetracycline resistance. Prototrophic ($CysG^+$) transductants were scored by replica-printing to minimal medium containing tetracycline. Selection for prototrophy eliminated potential candidates carrying *aroB* mutations polar on *dam*. The total number of Tet^r $CysG^+$ transductants obtained was 889. These were then examined for slow growth and/or abnormal colony morphology on green plates (Dam^- mutants of *E. coli* show

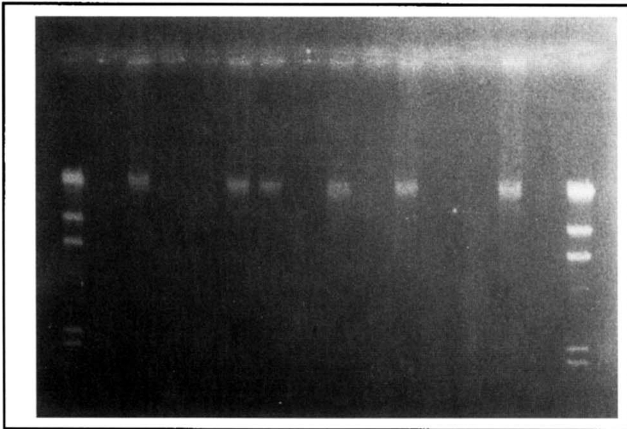

FIGURE 1.—Digestion of genomic DNAs by restriction enzymes with different responses to the methylation state of their targets (see the text for details). Lanes are as follows: 1–4, Dam$^+$ (LT2); 5–8, Dam$^-$ (SV3000); 9–12, Dam$^-$/pTP166 (SV3014). Lanes 1, 5 and 9 are controls of undigested DNA; lanes 2, 6 and 10 contain *Sau3*AI digestions; lanes 3, 7 and 11 contain *Mbo*I digestions; lanes 4, 8 and 12 contain *Dpn*I digestions. Lanes at both gel edges contain DNA size markers (*Hin*dIII-digested lambda DNA).

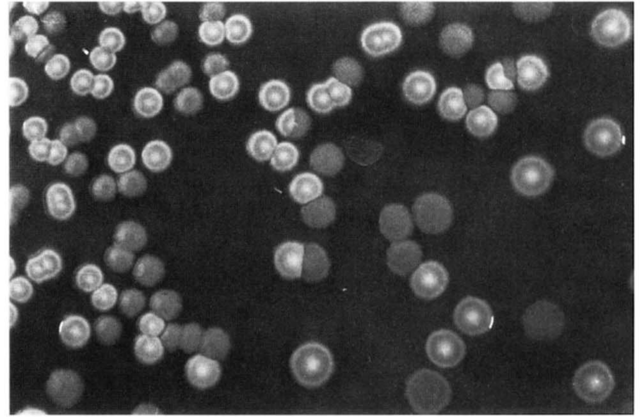

FIGURE 2.—A mixture of colonies formed by isogenic Dam$^+$ and Dam$^-$ strains of *S. typhimurium* (LT2 and SV3000, respectively) on green plates. The wild type forms glossy convex colonies, while the colonies formed by the Dam$^-$ strain are dull and flat.

abnormal cell morphology: see BARRAS and MARINUS 1989).

Twenty candidates forming abnormal colonies were physically analyzed by digestion of genomic DNA preparations with endonucleases *Dpn*I, *Sau*3AI and *Mbo*I. All these enzymes recognize the sequence 5′-GATC-3′, but *Mbo*I activity is blocked by Dam methylation, while *Dpn*I only cuts methylated DNA; *Sau*3AI cuts both methylated and umethylated DNA. Three independent isolates (each obtained from a different pool) proved to be Dam$^-$, as judged from their restriction patterns. These isolates were the origin of strains JC608, JC609 and JC610; their *dam* alleles were designated *dam-201, dam-202* and *dam-203,* respectively. The three strains looked identical in genetic and physical tests and their Southern hybridization patterns against a Tn*10* probe were identical (data not shown); thus they were probably generated by Tn*10d*Tet insertions in the same site. For further work, the allele *dam-201*::Tn*10* of strain JC608 was transduced to LT2, giving rise to strain SV3000 (see its DNA restriction pattern in Figure 1, lanes 5–8). This strain was used as the standard Dam$^-$ insertion mutant of *S. typhimurium.*

On green plates, Dam$^-$ mutants of *S. typhimurium* form flat, dull colonies that are easily distinguished from the convex, glossy colonies of the wild type (Figure 2). This distinct colony morphology is a reliable trait and can be used for strain construction.

For the generation of allele variants, a lysate grown on strain TT10425 was UV-irradiated and used to transduce SV3000. Kan^r transductants appeared at frequencies around 10^{-9} per p.f.u.; Kan^r transductants were scored for loss of tetracycline resistance and for the maintenance of phenotypes characteristic of Dam$^-$ mutants (flat colony shape, distinct restriction pattern of genomic DNA). One of the substitution isolates was the origin of strain SV3001.

Isolation of Tn*10d*Cam insertions linked to the *dam* locus: Pools of random Tn*10d*Cam insertions were made on strain SV3000; insertions linked to the the mutation *dam-201*::Tn*10d*Tet were detected by cotransduction, using strain LT2 as the recipient. Two independent insertions linked to the *dam* locus were obtained; the closest was *zhf-6304,* >90% linked to *dam* (see Figure 3).

Isolation and characterization of deletion mutants: Tetracycline-sensitive derivatives of strain SV3000 were isolated on Bochner-Maloy plates (BOCHNER *et al.* 1980; MALOY and NUNN 1981). Although the selection agar contains tryptone and yeast extract, *aroB* mutants are unable to form colonies on this medium unless supple-

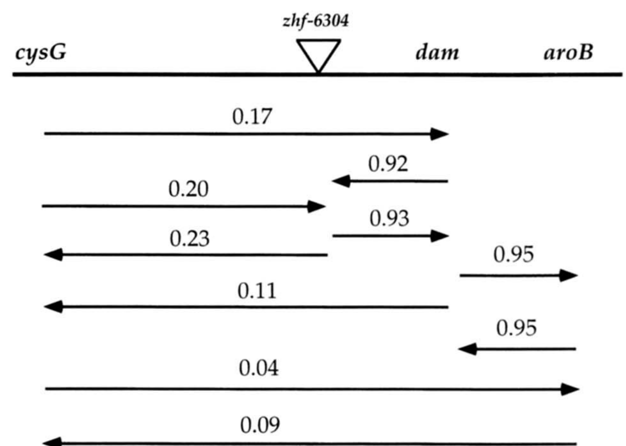

FIGURE 3.—Genetic map of the *cysG-dam-aroB* region of the *S. typhimurium* chromosome, constructed by P22-mediated transductional crosses.

mented with "aromatic mix". This selection permits the isolation of Tet^s derivatives carrying only small deletions, because their extent is constrained by *aroB* at one side and by the essential gene *trpS* at the other. One Tet^s derivative of strain SV3000 was the origin of strain SV3006; its deletion allele was named Δ*dam-204*. Genomic DNA from this strain was still able to hybridize against a *dam* probe (an *XbaI-PvuII* fragment of plasmid pTP166). However, a band shift was observed, suggesting that the *dam* gene had suffered a partial deletion (data not shown). Further evidence that Δ*dam-204* is a deletion allele was obtained during the study of a *lac* fusion repressed by Dam methylation (see below).

Complementation of *dam* mutations of *S. typhimurium* by the *E. coli dam*$^+$ gene: Plasmid pTP166 was transferred to strain TR5878 by transformation, selecting ampicillin resistance. The resulting strain, TR5878/pTP166, was used as donor to transduce pTP166 to various recipients in crosses mediated by P22 HT. When plasmid pTP166 was introduced in *S. typhimurium* Dam$^-$ mutants (*e.g.*, SV3000 or SV3006), it complemented the *dam* mutation: all the Amp^r transductants regained both the wild-type colony shape (not shown) and a wild-type pattern of digestion by endonucleases *DpnI*, *Sau3AI* and *MboI* (see lanes 10–12 in Figure 1).

Genetic mapping of *dam* mutations: Two- and three-factor crosses were carried out by P22 HT transduction; linkage of unselected markers was scored by replica-printing. Preliminary mapping of the *dam* locus was performed using the alleles *dam-201::*Tn*10d*Tet, *dam-202::*Tn*10d*Tet and *dam-203::*Tn*10d*Tet. Further work, including strain constructions for three-factor crosses, was carried out using the alleles *dam-201::*Tn*10d*Tet and Δ*dam-204*, as well as the linked markers *aroB542::*Tn*5*, *cysG439* and *zhf-6304::*Tn*10d*Cam. The resulting genetic map is shown in Figure 3. It must be noted that linkage between *aroB* and *dam* mutant alleles must be established using the antibiotic-resistance of a Tn*10* insertion in *dam*, because the *aroB542::*Tn*5* mutation causes a leaky Dam$^-$ phenotype (data not shown).

Viability of *dam* mutations in combination with other mutations: Viability tests involved two types of transductional crosses:

(1) Insertion alleles (*e.g.*, *recD541::*Tn*10d*Cam, *recF522::*Tn*5*, *recB503::*Tn*10*, and *recJ504::*Mu*dJ*) were transduced to an isogenic pair of Dam$^+$ and Dam$^-$ recipients. A given combination was judged inviable whenever the frequency of transductants was >1000-fold reduced. The results of these experiments, summarized in Table 2, indicate that *dam* mutations of *S. typhimurium* are inviable if combined with *recB* mutations, but not with *recD*, *recF* or *recJ*. Note a relevant difference between *S. typhimurium* and *E. coli*: in the latter, the combination *dam recJ* is inviable (PETERSON *et al.* 1985).

(2) A lysate grown on a *recA1 srl-203::*Tn*10d*Cam donor (TT11289) was used to transduce isogenic Dam$^+$ and Dam$^-$ recipients. The *srl* and *recA* loci are 50% linked (SANDERSON and ROTH 1983). Cotransduction of *recA* and *srl* was detected by scoring UV sensitivity. The combination *recA dam* was judged inviable because 300/300 Cam^r (Srl$^-$) transductants were UV^r. Thus, as in *E. coli* (PETERSON *et al.* 1985), the combination *recA dam* is inviable in Salmonella.

In *E. coli* the inviability of *dam* mutations in combination with certain recombination functions has been attributed to the need of recombination to repair double-strand breaks derived from MutHLS-catalyzed incisions (WANG and SMITH 1986). An analogous picture can be drawn for *S. typhimurium*, because the presence of *mutH*, *mutL* or *mutS* mutations in the Dam$^-$ recipient permitted the isolation of RecB$^-$ transductants in P22-mediated crosses (Table 2). RecA$^-$ Dam$^-$ strains can be likewise constructed if the recipient contains a *mutH* allele (data not shown).

Lack and overproduction of DNA adenine methylase cause hypermutability: Rates of spontaneous mutation to rifampicin resistance were compared by plating early stationary cultures on NB plates supplemented with rifampicin. The frequency of Rif^r mutants increased from 10^{-8} in the wild type to $9–15 \times 10^{-8}$ in Dam$^-$ strains. However, the highest mutation rates (>400-fold higher than the wild type) were observed when the *E. coli dam* gene was introduced into *S. typhimurium* on a multicopy plasmid. Thus lack of Dam methylase causes a milder hypermutability than Dam overproduction, suggesting that faster DNA remethylation perturbs mismatch repair more severely than the absence of methylation. Similar results have been reported for *E. coli* (MARINUS *et al.* 1984).

The mutation pattern of Dam$^-$ strains was examined using the *lacZ* allele collection constructed by CUPPLES and MILLER (1989). Six F' *lac pro*$^+$ episomes, each containing a known base substitution in the *lacZ* gene, were introduced in isogenic Dam$^+$ and Dam$^-$ strains of *S. typhimurium*. The six *lacZ* alleles cover all six possible base substitutions (CUPPLES and MILLER 1989); the genotypes of the strains used (SV3019–SV3030) are given in the strain list. The presence of a *dam* mutation in the background increased around eightfold the reversion rates of two alleles (*lacZ102* and *lacZ106*) but had little or no effect on the other members of the *lacZ* allele collection. These results indicate that the increase of Lac$^+$ reversion observed in a Dam$^-$ host is clearly biased toward transition mutations, as in *E. coli* (GLICKMAN 1979).

Effect of *dam* mutations on SOS induction: SOS activity was tested in *lexA*$^+$/*lexA::lacZ* merodiploids (strains SV1240, SV3007 and SV3008), constructed by using a Tn*5*-borne *lexA::lac* fusion (GARRIGA 1992). Measurements of β-galactosidase activity indicated that *dam* mutations of Salmonella, like their *E. coli* counterparts (PE-

TABLE 2

Viability of *S. typhimurium dam* mutations in combination with other mutations

Transduced allele	Recipient	Genotype of the recipient	EOT[a]
recB497::Mu*dJ*	LT2	*dam*$^+$	1
	SV3000	*dam-201*::Tn*10d*Tet	$<10^{-3}$
	SV3006	Δ*dam-204*	$<10^{-3}$
	SV3011	Δ*dam-204 mutL111*::Tn*10*	0.33
	SV3012	Δ*dam-204 mutS121*::Tn*10*	0.45
recB503::Tn*10*	SV3013	Δ*dam-204 mutH101*::Tn*5*	1.2
recD541::Tn*10*dCam	LT2	*dam*$^+$	1
	SV3000	*dam-201*::Tn*10d*Tet	1.51
recF522::Tn*5*	LT2	*dam*$^+$	1
	SV3000	*dam-201*::Tn*10d*Tet	0.21
recJ504::Mu*d*Cam	LT2	*dam*$^+$	1
	SV3000	*dam-201*::Tn*10d*Tet	0.49

[a] EOT (efficiency of transduction) is the ratio between the numbers of transductants obtained on a pair of isogenic strains.

TERSON *et al.* 1985), undergo moderate (two- to threefold) derepression of *lexA* transcription. Derepression was not only observed for *lexA;* plate tests with plasmid-borne *umuDC::lac* and *cea::lac* fusions (ELLEDGE and WALKER 1983; SALLES *et al.* 1987) also showed increased expression in a Dam$^-$ background (data not shown). Thus *S. typhimurium dam* mutations cause moderate SOS induction, like those of *E. coli* (PETERSON *et al.* 1985).

UV sensitivity assays: Dam$^-$ mutants of *E. coli* are slightly UV-sensitive at low UV doses (GLICKMAN *et al.* 1978), but their UV sensitivity increases with respect to wild type at higher UV doses (MARINUS and MORRIS 1974). We examined the UV sensitivity of *S. typhimurium* Dam$^-$ isolates and observed a difference with their *E. coli* counterparts: Dam$^-$ mutants of Salmonella are UV-resistant at both low and high UV doses (Figure 4). Given the differences in UV mutability between *E. coli* and Salmonella (reviewed by EISENSTADT 1987) and the presence of *umuDC*-like genes in the *S. typhimurium* virulence (sometimes called "cryptic") plasmid (NOHMI *et al.* 1991), we examined the possibility that the UV resistance of *S. typhimurium* Dam$^-$ mutants might involve plasmid-borne function(s). For this purpose, we constructed a Dam$^-$ derivative of a strain cured of the virulence plasmid and compared its UV sensitivity with that of the parental strain, TR5527. Both UV sensitivity curves were similar and roughly identical to those of Dam$^+$ and Dam$^-$ LT2 derivatives shown in Figure 4. Thus Dam$^-$ strains of *S. typhimurium* may be intrinsically resistant to UV radiation. Or, if alternative functions exist, they must lie in the chromosome and not in the virulence plasmid.

Effect of *dam* mutations on duplication segregation: Duplication segregation was examined using a test for homogenote formation formally similar to that of MARINUS and KONRAD (1976); the only difference is

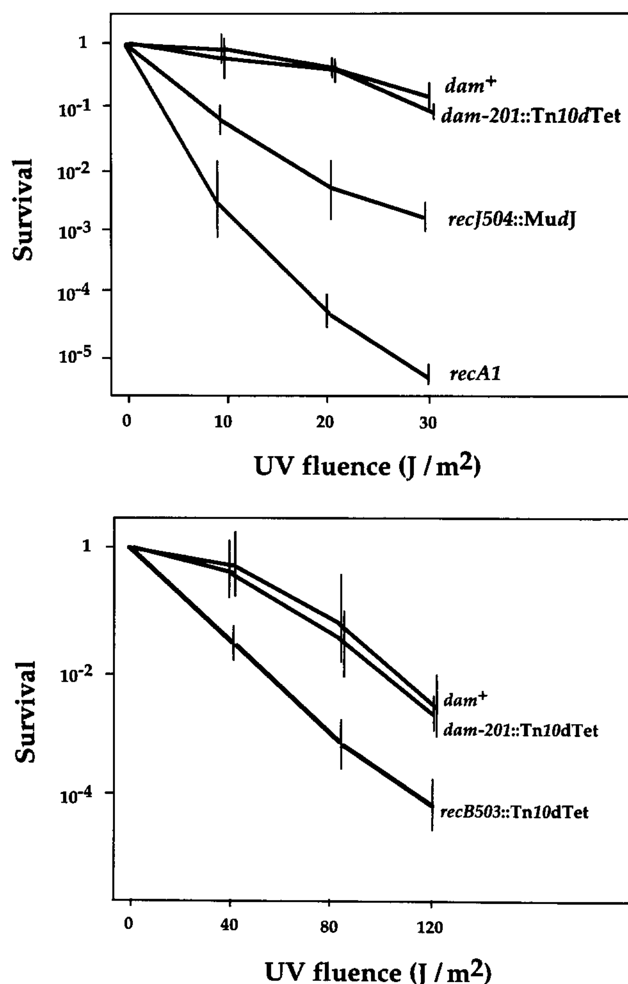

FIGURE 4.—UV sensitivity curves of Dam$^+$ and Dam$^-$ strains of *S. typhimurium* (LT2 and SV3000, respectively). To illustrate the UV sensitivity levels, three isogenic strains carrying recombination mutations are also included (TT15278, TT10838 and SV1223).

TABLE 3

Effect of *dam* mutations on the segregation of chromosomal duplications

Strain	Genotype	% of segregants[a]
SV3004	DUP [*trp-2482* *Mud1-8* *hisD9953*]	67
SV3005	DUP [*trp-2482* *Mud1-8* *hisD9953*] *dam-201*::Tn*10*dTet	97
SV3010	DUP [*trp-2482* *Mud1-8* *hisD9953*] Δ*dam-204*	99
SV3031	DUP [*trp-2482* *Mud1-8* *hisD9953*] *mutH101*::Tn*5*	33
SV3015	DUP [*trp-2482* *Mud1-8* *hisD9953*] Δ*dam-204 mutH101*::Tn*5*	40

[a] Percentage of Amp^s colonies; average of three experiments.

that we used chromosome merodiploids instead of F-prime heterogenotes. Isogenic Dam$^+$ and Dam$^-$ pairs of strains carrying Mu*d*-induced duplications with known endpoints were grown nonselectively in NB until saturation. Absence of antibiotic selection permits segregation of the Mu*d*-held duplication (HUGHES and ROTH 1985). Colonies were isolated on NB plates and haploid segregants lacking the Mu*d*-encoded antibiotic resistance (Amp^s) were detected by replica-printing (FLORES and CASADESUS 1995).

Table 3 shows that duplication segregation increases in a Dam$^-$ background; the increase can be suppressed by a *mutH* mutation. These results suggest that *dam* mutations enhance duplication segregation via mismatch repair, probably because the MutH endonuclease introduces nicks or double-stranded breaks in unmethylated DNA. Because the activated form of MutH can cleave both DNA strands at an unmethylated 5'-GATC-3' site (AU *et al.* 1992), rare mismatches generated during DNA replication can trigger MutHLS activity. The free DNA ends generated can then be used to initiate homologous recombination, as proposed for *E. coli* (MARINUS and KONRAD 1976).

A genetic screening for the detection of *lac* transcriptional fusions regulated by DNA adenine methylation: Kan^r transductants generated by Mu*dJ* insertion were classified according to their Lac phenotype; 100–500 insertions of the same class (Lac$^+$ or Lac$^-$) were then pooled and lysed with P22 HT. The lysates were used to transduce a Dam$^-$ recipient (SV3000), selecting Kan^r transductants on indicator plates. This procedure can be expected to permit the detection of fusions in genes whose transcription is regulated by DNA adenine methylation:

(1) Lac$^-$ isolates that turn Lac$^+$ in a Dam$^-$ background carry fusions putatively repressed by Dam methylation.

(2) Lac$^+$ isolates that turn Lac$^-$ in a Dam$^-$ background carry fusions putatively activated by Dam methylation.

To be classified as isolates carrying a fusion regulated by Dam methylation, candidates are required to pass three additional tests:

(1) A "backcross" in which the original Dam$^+$ isolate is made Dam$^-$ by transduction of the insertion *dam-201*::Tn*10d*Tet. This test intends to confirm that the change in color is caused by the *dam* mutation (and not by another mutation carried by the Dam$^-$ recipient).

(2) Reconstruction crosses, in which the fusion is transduced to isogenic Dam$^+$ and Dam$^-$ derivatives (LT2 and SV3006, respectively). Reconstruction in recipients different from the pair of original strains intends to confirm that the fusion is regulated by Dam methylation irrespective of the background of the recipient.

(3) Complementation with a cloned *dam*$^+$ allele (carried on plasmid pTP166). This test can confirm that the fusion is regulated by Dam methylation (and not by a gene located downstream of *dam*). This test is necessary whenever the *dam* alleles used are potentially polar (insertions or out-of-frame deletions).

Genetic characterization of a fusion repressed by Dam methylation: Among the candidates obtained with the screening described in the former section, a *lac* fusion repressed by Dam methylation was chosen for further study. Dam$^+$ isolates carrying this fusion form white colonies on MacConkey lactose, while Dam$^-$ isolates form red colonies. In a Dam$^-$ host, the presence of plasmid pTP166 causes repression of the fusion to levels similar to the wild type (data not shown). Histograms of β-galactosidase activities are shown in Figure 5. The activity of the fusion is 10-fold higher in a Dam$^-$

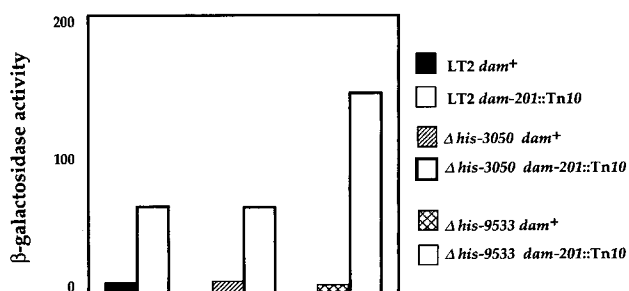

FIGURE 5.—β-galactosidase activities of isogenic pairs of Dam$^+$ and Dam$^-$ strains carrying the *zzv-6306* Mu*dJ* fusion in different backgrounds: *his*$^+$ *dam*$^+$ (SV3003), *his*$^+$ *dam*$^-$ (SV3069), Δ*his-3050 dam*$^+$ (SV3070), Δ*his-3050 dam*$^-$ (SV3071), Δ*his-9533 dam*$^+$ (SV3072) and Δ*his-9533 dam*$^-$ (SV3073).

background, a derepression level similar to that of the IS*10* transposase gene (ROBERTS *et al.* 1985) and higher than those of other Dam-repressed genes such as *glnS*, *trpR* and *sulA* (MARINUS 1985, 1987a,b; PETERSON *et al.* 1985). The fusion is not inducible by DNA damage, as indicated by treatments with mitomycin C and nalidixic acid (data not shown).

Isolates carrying the fusion in a Δ*dam-204* background are stable and do not segregate Lac^- colonies or sectors. In contrast, isolates carrying the fusion in a *dam-201*::Tn*10d*Tet background yield colonies containing Lac^- sectors at low frequency (<1%). Sectored colonies are not found if the plates contain tetracycline. These results suggest that the *dam-201*::Tn*10d*Tet insertion can undergo a low but detectable rate of excision; in fact, transposase-independent Tn*10* excision has been shown to be enhanced in Dam^- mutants of *S. typhimurium* (HAFNER and MACPHEE 1991). Most Lac^- sectors seem to be formed by Dam^+ revertants, as judged from the colony shape of purified Lac^- isolates and from their ability to inherit a *recB* mutation (data not shown). These observations provide further evidence that the allele Δ*dam-204* cannot revert (and thus is a deletion allele).

The fact that the fusion had been isolated in strain TT1704, the usual recipient for transductional delivery of Mu*dJ* (HUGHES and ROTH 1988), prompted a second, unexpected observation: in the TT1704 background, the ratio of β-galactosidase activities found in Dam^- and Dam^+ hosts is 26. This result suggests that TT1704 carries a second mutation that activates the fusion in the absence of Dam methylation. This (hypothetical) mutation has no effect in a Dam^+ background (Figure 5). The deletion carried by TT1704 (Δ*his-9533*) is nontransducible by P22; thus it must exceed the packaging capacity of the P22 capsid, 43 kb (CASJENS and HAYDEN 1988). Smaller deletions such as Δ*his-3050* do not increase the expression of the fusion (Figure 5). Thus we hypothesize that a locus located near the histidine operon may corepress the expression of the fusion.

For mapping with the "locked-in" Mud-P22 procedure (BENSON and GOLDMAN 1992), a Tn*10d*Tet element was introduced by homologous recombination in the *lacZ* gene of the Mu*dJ*-generated fusion. For this cross, a P22 lysate grown on strain TT16716 was UV-irradiated and used to transduce SV3003, selecting tetracycline resistance on X-gal plates. Four Tet^r Lac^- transductants were obtained. All carried a Tn*10* insertion 100% linked to the resident Mu*dJ* element. One of these isolates was the origin of strain SV3074.

Attempts to map the Tet^r insertion (and thus the Mu*dJ* fusion) with the locked-in Mud-P22 procedure (BENSON and GOLDMAN 1992) did not provide patches of Tet^s transductants, suggesting that the locus might not map on the chromosome. Evidence that the fusion mapped on the *S. typhimurium* virulence plasmid was

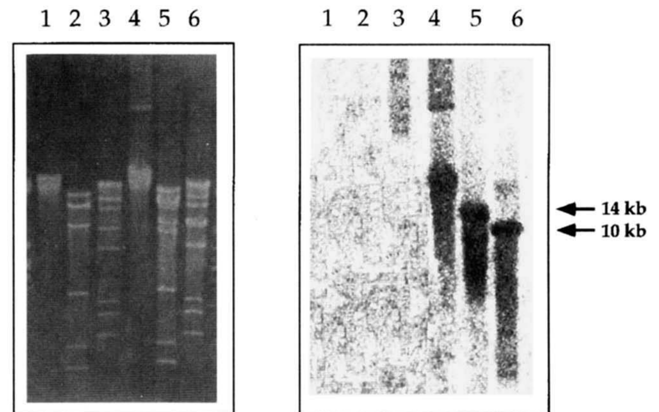

FIGURE 6.—(A) Agarose gel electrophoresis of virulence plasmid DNA preparations from strains LT2 and SV3003. Lanes are as follows: (1) virulence plasmid from LT2, undigested; (2) virulence plasmid from LT2, *Eco*RI-digested; (3) virulence plasmid from LT2, *Hin*dIII-digested; (4) virulence plasmid from SV3003, undigested; (5) virulence plasmid from SV3003, *Eco*RI-digested; (6) virulence plasmid from SV3003, *Hin*dIII-digested. (B) Southern hybridization of the DNA fragments shown in A, using the *Hin*dIII fragment of plasmid pIZ53 as a probe; this fragment contains the Tn*5* kanamycin resistance gene (which is the same Kan^r determinant carried by the Mu*dJ* element).

obtained in transductional crosses: when the original Mu*dJ* element or its Tn*10*-containing derivative were transduced to the wild-type LT2, transductants were obtained at frequencies near 10^{-5} per p.f.u. In contrast, no transductants were obtained when the recipient was a strain cured of the virulence plasmid (TR5527).

Physical mapping confirmed that the Mu*dJ*-generated fusion was located on the *S. typhimurium* virulence plasmid. DNA preparations of the latter were digested with *Eco*RI or *Hin*dIII and hybridized against the kanamycin gene of the Mu*dJ* element. The results confirmed that the fusion is plasmid-borne: the Mu*dJ* probe hybridized with a ~14-kb *Eco*RI fragment (Figure 6B, lane 5), with a ~10-kb *Hin*dIII fragment (Figure 6B, lane 6), and with undigested virulence plasmid DNA (Figure 6B, lane 4). The fusion has been named *zzv-6306*::Mu*dJ*.

DISCUSSION

A *S. typhimurium* point mutant lacking DNA adenine methylase was described a decade ago (RICHTIE *et al.* 1986) and specific aspects of Dam methylation in Salmonella have been since then investigated (RICHTIE *et al.* 1988; HAFNER and MACPHEE 1991). Although the pioneering relevance of these studies must be emphasized, their use of point mutants can be viewed as a potential source of problems. Because *dam* mutations are deleterious and highly pleiotropic, Dam^- mutants are prone either to revert or to accumulate partial suppressors (MACGRAW and MARINUS 1980); thus periodical reconstruction of Dam^- mutants is highly advisable.

This can be readily achieved by the use of insertion alleles such as the Tn*10d*Tet insertions described in this work. In turn, certain operations of strain construction are made easier by the use of deletion alleles that lack the Tn*10*-encoded tetracycline resistance. Whenever necessary, Dam$^-$ deletion strains can be reconstructed using nearby insertions such as *zhf-6304*::Tn*10d*Cam. For strain construction, one useful phenotype of Dam$^-$ mutants of *S. typhimurium* is their abnormal colony morphology on green plates. Like other phenotypes of Dam$^-$ strains, the unusual aspect of their colonies tends to disappear upon repeated subculture, but it is a reliable trait to score for Dam$^-$ transductants. Confirmation of the *dam* genotype may require either a genomic DNA digestion test or a compatibility assay with *recA* or *recB* mutations (see below).

Tn*10d*Tet insertions in the *dam* locus of *S. typhimurium* map at 75 min, as previously reported (RICHTIE *et al.* 1986). Cotransductional mapping shows that the *dam* locus is 10% linked to *cysG* and 95% linked to *aroB;* these data suggest a position identical to that of the *E. coli dam* gene (MARINUS 1973; LYNGSTADAAS *et al.* 1995). The structure of the *dam* gene itself is likely to be conserved, because many (if not all) phenotypes of Salmonella Dam$^-$ mutants are efficiently complemented by a plasmid containing the *E. coli* wild-type *dam* gene. A corollary is that the phenotypes that can be complemented are unequivocally caused by lack of DNA adenine methylase (and not by a polar effect of the *dam* mutation on downstream genes).

Deletion alleles were obtained by selecting Tet^s derivatives of Tn*10d*Tet insertions in *dam.* Deletions that remove genetic material from *dam* toward *cysG* are necessarily small because of the presence of the essential gene *trpS* (LYNGSTADAAS *et al.* 1995; SANDERSON *et al.* 1995). At the other side of *dam,* the extent of deletions can be constrained by taking advantage of the presence of the *aroB* gene: if Tet^s derivatives are obtained on plates lacking an aromatic mix supplement, only AroB$^+$ Tet^s derivatives are obtained. The advantage of these constraints is the yield of deletions (such as Δ*dam-204* and others) that remove little genetic material around *dam* and thus are safe to identify the consequences of loss of the Dam function (and not the combined effects of multigenic losses).

Attempts to construct double mutants containing a *dam* mutation and another mutation affecting recombination and/or repair has indicated that the combinations *recA dam* and *recB dam* are inviable, while the combinations *recD dam, recF dam* and *recJ dam* are viable. These data indicate that viability of Dam$^-$ strains of *S. typhimurium* requires certain recombination functions (RecA and RecBC) while others are dispensable (RecD, RecJ and RecF). By analogy with *E. coli,* one may interpret this to mean that Dam$^-$ strains suffer a higher incidence of MutH-catalyzed double strand breaks (WANG and SMITH 1986). This view is supported by the observation that Dam$^-$ RecA$^-$ and Dam$^-$ RecB$^-$ mutants of *S. typhimurium* are viable in the presence of mutations that eliminate mismatch repair such as *mutH, mutL* or *mutS.* Thus the RecA and RecBCD functions seem to be involved in the repair of the double-strand breaks typical of Dam$^-$ strains of Salmonella. For practical purposes, the incompatibility between *recB* and *dam* mutations permits a rapid identification test: any putative Dam$^-$ construct can be identified as such if it fails to inherit a *recB* insertion allele in a P22-mediated transductional cross (well-characterized insertions in *recA* are not available in *S. typhimurium*).

An important difference between Dam$^-$ mutants of *E. coli* and Salmonella concerns RecJ function: Dam$^-$ RecJ$^-$ mutants are viable in *S. typhimurium* but not in *E. coli* (PETERSON *et al.* 1985). A recent study has suggested that repair of double-strand breaks in methylation-deficient strains of *E. coli* requires a RecBC-dependent pathway that includes RecJ (PETERSON and MOUNT 1993). In contrast, our data indicate that RecJ is not necessary for viability of Dam$^-$ strains of Salmonella, at least in an SbcB$^+$ background. Although the difference is surprising, one should keep in mind that the RecJ product may play slightly different roles in *E. coli* and Salmonella (MAHAN *et al.* 1992).

Another unexpected difference between Dam$^-$ mutants of *E. coli* and Salmonella is UV sensitivity: unlike *E. coli, S. typhimurium* Dam$^-$ mutants are not UV-sensitive (not even at high UV doses: see MARINUS and MORRIS 1973; GLICKMAN *et al.* 1978). This observation suggests that methyl-directed DNA repair does not play a significant role in the repair of UV damage in Salmonella. This observation is supported by the observation that *mut* alleles do not cause UV sensitivity (SHANABRUCH *et al.* 1981). However, we were surprised by the difference between *E. coli* and Salmonella and considered the possibility that Salmonella might have alternative repair functions. An obvious genetic entity that might harbor those hypothetical functions is the *samAB*-containing virulence plasmid (NOHMI *et al.* 1991). However, Dam$^-$ mutants proved to be still UV^r in the absence of the virulence plasmid. Thus, if alternative functions exist, they must map elsewhere.

Aside from the differences discussed above, Dam$^-$ mutants of *S. typhimurium* share with those of *E. coli* many relevant phenotypes: (1) increased incidence of spontaneous mutations, biased toward transitions (GLICKMAN 1979); (2) severe hypermutability (MARINUS *et al.* 1984); (3) moderate SOS induction (PETERSON *et al.* 1985) and (4) enhanced duplication segregation, which can be suppressed by mutations that eliminate mismatch repair (MARINUS and KONRAD 1976). The balance of analogies and differences indicates that Dam methylation performs similar roles in *E. coli* and Salmonella. Moreover, the ability of the *E. coli dam* gene to

complement *S. typhimurium dam* mutations suggests that Dam methyltransferase may be a function highly conserved among both taxa. On the other hand, the differences found are less surprising if one considers that the genera Escherichia and Salmonella diverge in other aspects of recombination and repair (EISENSTADT 1987).

Genes regulated by Dam methylation have been traditionally discovered by reverse genetics, after the presence of 5′-GATC-3′ sites in or near the promoter (MARINUS 1987; NOYER-WEIDNER and TRAUTNER 1993). In contrast, classical genetic strategies like the *lac* fusion screening described above can identify novel, uncharacterized loci regulated by Dam methylation. Our screening has two caveats: (1) it relies on the use of unmethylated DNA that, in general, is a nonphysiological condition; thus it might give rise to artefacts whenever hemimethylation and unmethylation are not equivalent signals; (2) small differences in transcriptional activity may not be easily seen on plate tests. Despite these potential limitations, the screening has already proved useful to detect a locus repressed by Dam methylation, located in the *S. typhimurium* virulence plasmid.

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