JOURNAL OF BACTERIOLOGY, Nov. 1989, p. 5776–5782
0021-9193/89/115776-07$02.00/0

Vol. 171, No. 11

Cloning of *Salmonella typhimurium* DNA Encoding Mutagenic DNA Repair

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Received 8 June 1989/Accepted 5 August 1989

Mutagenic DNA repair in *Escherichia coli* is encoded by the *umuDC* operon. *Salmonella typhimurium* DNA which has homology with *E. coli umuC* and is able to complement *E. coli umuC122*::Tn*5* and *umuC36* mutations has been cloned. Complementation of *umuD44* mutants and hybridization with *E. coli umuD* also occurred, but these activities were much weaker than with *umuC*. Restriction enzyme mapping indicated that the composition of the cloned fragment is different from the *E. coli umuDC* operon. Therefore, a *umu*-like function of *S. typhimurium* has been found; the phenotype of this function is weaker than that of its *E. coli* counterpart, which is consistent with the weak mutagenic response of *S. typhimurium* to UV compared with the response in *E. coli*.

Mutagenesis of *Escherichia coli* induced by many chemical and physical agents requires expression of the *umuDC* operon (2, 17, 48, 49). These genes encode a DNA repair activity which increases the tolerance of the cell for DNA damage but which does so with an intrinsic probability of creating a mutational change in DNA sequence (for reviews, see references 53 and 55). This mutagenic DNA repair function can also be provided by many resistance transfer plasmids (28, 37; for a review, see reference 50) which carry genes with similar structure and regulation to *umuDC* (10–13, 18, 34, 35, 51).

Expression of *umuDC* is increased as part of the induction of the multigene SOS response to perturbations of DNA structure or replication (2, 12, 44). Derepression occurs through an activated form of RecA protein which serves as a cofactor for an autocleavage reaction of the LexA protein repressor (8, 20, 46). The UmuD protein produced then undergoes autocleavage by a similar interaction with activated RecA protein. UmuD protein becomes proficient in mutagenesis only after this cleavage reaction and the production of its carboxy-terminal fragment (7, 30, 43). The exact role of the UmuD and UmuC proteins in mutagenesis is still unknown, but it is believed that they may allow DNA replication to resume beyond lesions (5).

The basic regulatory elements of the SOS response are therefore RecA and LexA proteins. There is considerable evidence that similar activities are well conserved in relatives of *E. coli* such as *Salmonella typhimurium* (33, 36, 38, 40, 41), as well as in unrelated species such as *Bacillus subtilis* (21 and references therein; 22). Many of the activities induced by SOS induction of *E. coli* are also produced in these other species. Even though *S. typhimurium* and *E. coli* share highly conserved mechanisms of SOS regulation, there are differences between their responses to DNA damage. While many studies have demonstrated chemically induced mutagenesis in *S. typhimurium* (1), several reports suggest that UV induces a lower level of mutagenesis in *S. typhimurium* than in *E. coli* (15, 23, 31, 32). There are also some studies in which no UV-induced mutagenesis was detected (40, 45). The related process of Weigle reactivation of bacteriophage is again less efficient in *S. typhimurium* than in *E. coli* (52). The limiting factor in *S. typhimurium* appears to be *umuDC*-like activity, because mutagenesis is restored by plasmids carrying *E. coli umuDC* (15) or analogous genes such as *mucAB* (23, 27, 29). The reduced mutagenesis and Weigle reactivation of *S. typhimurium* are therefore reminiscent of *E. coli umuC* mutants (17, 54). Furthermore, *S. typhimurium* recombinants which have incorporated the *E. coli trp* region become more UV mutable, suggesting that they have also acquired the *E. coli umuDC* operon which maps close by (45). Similarly, an increase in mutagenesis is exhibited by *S. typhimurium* strains which carry an *E. coli umuD*$^+$ *C* mutant plasmid but not by those which carry a *umuD* mutant *C*$^+$ plasmid (15). The latter point was presented as evidence that *S. typhimurium* has a *umuC* gene but lacks *umuD* and that mutability is lower because the operon is incomplete. However, the complementation data of *umuD* plasmids in both *E. coli* and *S. typhimurium* are complex. *umuD*$^+$ plasmids also increase the mutability of wild-type *E. coli* strains (S. Sedgwick, unpublished observation), and therefore any effect of these plasmids in *S. typhimurium* need not be indicative of a *umu* genotype. Previous attempts to identify the *umu* genes of *S. typhimurium* by nucleotide hybridization were not successful (40), but this is not conclusive, since the *umuDC*, *mucAB*, and *impAB* systems do not cross-hybridize and yet the sequences of *mucAB*, *impAB*, and *umuDC* share significant homology (34, 50).

In order to resolve the status of the *umuDC* operon in *S. typhimurium*, we have made and screened an *S. typhimurium* genomic library for sequences which will complement *E. coli umu* mutations. In this paper we describe the isolation and characterization of such a clone.

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacteria used in this study are listed in Table 1. The multicopy plasmids pSE117 and pLM207 (26), which carry the *E. coli umuDC* operon, originated from G. Walker and were kindly provided by M. Blanco and B. Bridges. Plasmid pMH2532 carries approximately 4 kilobase pairs (kbp) of *S. typhimurium* DNA and was constructed in the course of this study.

Media and culture. Luria-Bertani agar and broth were used for routine bacterial culture (24) and supplemented, when necessary, with 50 μg of ampicillin per ml or 50 μg of kanamycin per ml for plasmid selection and maintenance. UV-induced reversion of *his4* mutants to His$^+$ used semien-

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TABLE 1. Bacterial strains

Species and strain	Genotype	Source (reference)
E. coli		
AB1157	*xyl-5 mtl-1 galK2* λ-rac⁻ *rpsL31 kdgK51* Δ(*gpt-proA*) *62 lacY1 tsx-33 supE44 thi-1 leuB6 hisG4 mgl-51 arg-3 rfbD1 ara-14 thr-1*	Mill Hill collection (16)
GW2100	AB1157 *umuC*::Tn*5*	G. Walker (12)
MH1300 AB1157	*srl*::Tn*10 recA1 umuC*::Tn*5*	This study
DH5	F⁻ *endA1 hsdR17* (r_K^- m_K^+) *thi-1* λ⁻ *recA1 gyrA96*	Mill Hill collection (14)
TK612	AB1157 *uvrA umuD44 supE44 relA1*	B. Bridges (17)
S. typhimurium		
LT2	Wild-type	L. Bullas
TA2659	*hisG428* Δ(*gal chl uvrB bio*)	B. Ames (19)
LB5010		L. Bullas (6)
NCTC 73	Natural isolate	NCTC[a]
NCTC 74	Natural isolate	NCTC
NCTC 5710	Natural isolate	NCTC
NCTC 8298	Natural isolate	NCTC
NCTC 8392	Natural isolate	NCTC
NCTC 10413	Natural isolate	NCTC

[a] NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England.

riched plates containing the following: Davis & Mingioli salts (9); 1% (wt/vol) glucose; 25 μg each of proline, threonine, arginine, and leucine per ml; 5 μg of thiamine per ml; 2.5% (vol/vol) Oxoid agar no. 2; and 1.5% (wt/vol) Difco Bacto agar as solidifier). Dilutions were made in 10 mM $MgSO_4$. All cultures were grown at 42°C to avoid a possible cold-sensitive phenotype which multicopy plasmids encoding other known mutagenic DNA repair genes confer (26). All antibiotics were from Sigma Chemical Co.

UV irradiation. UV radiation came from low-pressure mercury vapor lamps. Incident doses were determined with a UVX radiometer (Ultraviolet Products Inc., San Gabriel, Calif.) and were in the range of 0.05 to 1 J m^{-2} sec^{-1}. Mid-log-phase cells (3-ml samples) were washed and suspended in 10 mM $MgSO_4$ and irradiated in 50-mm plastic tissue culture dishes with regular stirring. UV survival and UV-induced reversion to histidine prototrophy were assayed as described previously (39). All plates were incubated at 42°C for 5 days prior to counting.

DNA methodology. Plasmid DNA was extracted from bacteria by the method of Birnboim and Doly (3). Genomic DNA from *S. typhimurium* LT2 was prepared by the method of Marmur (25). Restriction enzyme fragment probes for nucleotide hybridization were extracted from agarose by using Geneclean (Bio 101 Inc.). They were radioactively labeled by using the Multiprime Labelling Kit from Amersham International PLC and [α-^{32}P]thymidine from Dupont, NEN Research Products, Boston, Mass. Nucleic acid hybridization was performed as previously described by Sedgwick et al. (42) with the solutions described by them, except that filters were washed twice for 10 min in 5× SSCPE (0.75 M NaCl, 0.075 M trisodium citrate, 44 mM NaH_2PO_4, 5 mM EDTA). Hybridization and washing were performed at 60 or 65°C. Restriction endonucleases and phage T4 DNA ligase were obtained from Bethesda Research Laboratories, Inc. All other techniques used were as described by Maniatis et al. (24).

RESULTS

Cloning strategy. The genomic DNA of *S. typhimurium* which hybridized weakly to *E. coli umu* DNA was first mapped by Southern hybridization. From this mapping information, a subgenomic library of *S. typhimurium* DNA was constructed in pBR322. This library was then screened for functional complementation of *E. coli umuC* mutants. Details of these stages are as follows.

Identification of *S. typhimurium* sequences with *umu* homology. Multiple restriction enzyme digests of *S. typhimurium* genomic DNA were subjected to Southern hybridization with probes containing *E. coli umuC* or *umuD* sequences. The *umuC* probe was a 0.91-kbp *Bam*HI-*Sal*I internal fragment of *umuC* (18, 44), and it makes up 73% of the complete *umuC* sequence. Hybridization to the control track of *E. coli* DNA was strongest, even though the amount of *E. coli* DNA was used was only 1/10 the amount of *S. typhimurium* used. Even so, hybridization to *S. typhimurium* gave discrete bands (Fig. 1a). An identical filter was probed with a 0.71-kbp *Bgl*II-*Hin*cII fragment containing the 5′ end of *umuD* and some upstream sequences (18, 44) (Fig. 1b). It is evident from the identical restriction fragment patterns in Fig. 1a and b that the *umuD* probe hybridized to the same *S. typhimurium* restriction fragments as a *umuC* fragment did. The same hybridization patterns arising with probes made from closely linked *E. coli umu* sequences strongly indicated that hybridization was specific and that a *umu*-like gene(s) exists in *S. typhimurium*.

The hybridization signal with the *umuD* probe was much weaker than that with the *umuC* probe (Fig. 1a and b). When both probes were radiolabeled to the same extent, the *umuD* blot required approximately 20 times the length of exposure to generate the same hybridization signal intensity as did the *umuC* blot. Furthermore, *umuD* hybridization was done at 60°C rather than at the more stringent 65°C used for *umuC* hybridization. Therefore, *S. typhimurium* does have homology with *E. coli umuDC*, but this homology is much greater with *umuC* than with *umuD*.

The restriction fragment pattern from Fig. 1 and similar experiments with other restriction endonucleases were used to construct a restriction map of the putative *S. typhimurium umu*-like region (Fig. 2). A comparison of this map with the published restriction maps of the *E. coli umuDC*, *mucAB*, and *impCAB* operons suggests that the sequence of the putative *S. typhimurium umu* region differs from those of the other UV-protective and mutation-enhancing operons. Consequently, the sequence is not a directly transplanted *E. coli* gene or spurious *E. coli* contamination.

Cloning *S. typhimurium umu*-like genes. The *S. typhimurium* sequence identified above, which hybridized to the *E. coli umu* probes, was contained within a 3.9-kb *Eco*RI-*Bam*HI genomic fragment (Fig. 2). A subgenomic library containing approximately 4-kb *Eco*RI-*Bam*HI *S. typhimurium* sequences was constructed by ligating size-fractionated fragments of genomic DNA into *Eco*RI-*Bam*HI-cut pBR322. The ligation mix was expressed in *E. coli* DH5. Growth was at 42°C to avoid the possible cold sensitivity that other mutagenic DNA repair genes in multiple-copy vectors are known to confer (26). Approximately 4,000 transformant colonies were pooled, and their plasmid DNAs were extracted. These plasmid DNAs were then used to transform *E. coli* GW2100 *umuC122*::Tn*5*. A total of 1,496 Ampicillin-

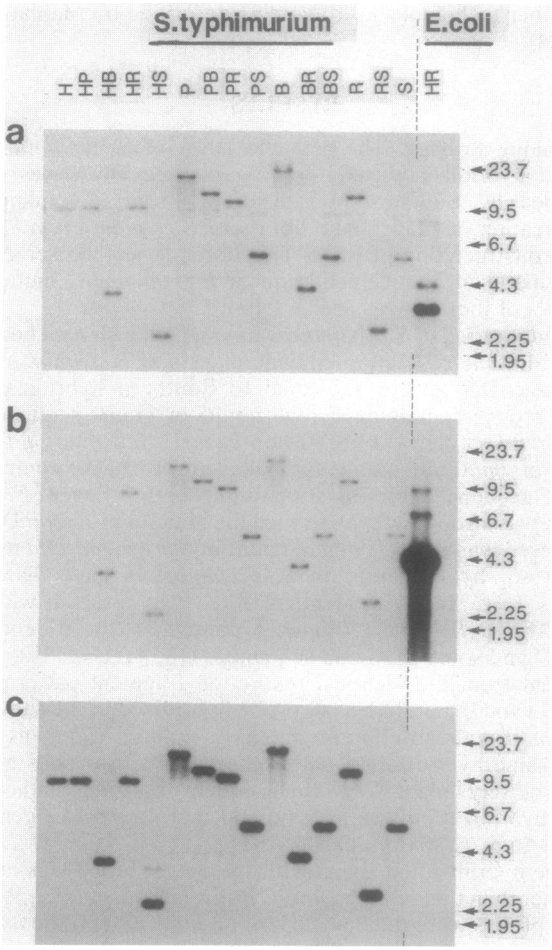

FIG. 1. Southern hybridization analysis of *S. typhimurium* genomic DNA with the following probes: *E. coli umuC*, a 0.91-kbp *Bam*HI-*Sal*I fragment of pSE117 (a); *E. coli umuD*, a 0.71-kbp *Bgl*II-*Hin*cII fragment of pLM207 (b); *S. typhimurium umuST*, a 2.2-kbp *Hin*dIII-*Sal*I fragment of pMH2532 (Fig. 2) (c). Hybridization temperatures were 65°C (a), 60°C (b), and 65°C (c). Exposure times were 3 h (a), 84 h (b), and 5 h (c). Restriction endonucleases: H, *Hin*dIII; P, *Pst*I; B, *Bam*HI; R, *Eco*RI; S, *Sal*I. Approximately 1 μg of *S. typhimurium* DNA was digested per track. *E. coli* DNA (0.1 μg) digested with *Hin*dIII-*Eco*RI served as a control. Molecular weights (kilobase pairs) are indicated on the right.

resistant transformants of strain GW2100 were screened for UV-induced mutability as an indicator for complementation of their *umuC* mutations. Each transformant was individually picked and patched in 10-mm^2 squares onto semienriched plates to test for reversion to His$^+$. The semienriched plates were irradiated with UV at 15 J m^{-2} and incubated at 42°C for approximately 7 days. All plates were scanned for patches which contained several His$^+$ revertant colonies, which could indicate possible complementation of the nonmutable *umuC122*::Tn*5* mutation. Colonies from patches which had an elevated number of His$^+$ revertants were repicked from the master plates and retested for UV-induced mutability. Twelve colonies were selected from this screen.

Confirmation of *umuC*-like properties. Three of the twelve clones complementing *E. coli umuC122*::Tn*5* (clones 1, 2, and 4) were arbitrarily chosen for further investigation, with an additional, randomly chosen clone which did not show any increase in the number of His$^+$ revertants. Clones 1, 2, and 4 showed increases from 3, 3, and 7 His$^+$ revertants per patch before irradiation to 28, 18, and 26 revertants after UV irradiation at 15 J m^{-2}, respectively. The control, clone 3, showed no UV-induced reversion increase over the one to three spontaneous revertants present per patch. Restriction digests of the four clones were subjected to Southern hybridization by using the internal fragment of *E. coli umuC* as a probe (Fig. 3). The complementing clones, clones 1, 2, and 4, hybridized with this *umuC* probe, and overexposure of this same filter (not shown) revealed that they were most likely recombined versions of one another. This is perhaps not surprising, given that the host bacteria were *recA*$^+$ and that *umu* plasmids may have a selective disadvantage (26). Clones 2 and 4 showed multiple banding patterns, while clone 1 gave exactly the restriction pattern predicted from the earlier restriction mapping of *S. typhimurium* genomic sequences (Fig. 2). Clone 3, which did not appear to complement the *umuC* defect of the host strain, did not hybridize to the *E. coli umuC* probe. Thus, the plasmids which gave functional complementation of the *umuC122*::Tn*5* defect of *E. coli* GW2100 specifically hybridized to an *E. coli umuC* probe. Furthermore, the restriction site map of the cloned material in one of these plasmids was identical to the map of the putative *umu*-like genomic region of *S. typhimurium* determined by Southern hybridization (Fig. 2) within the cloned sequence.

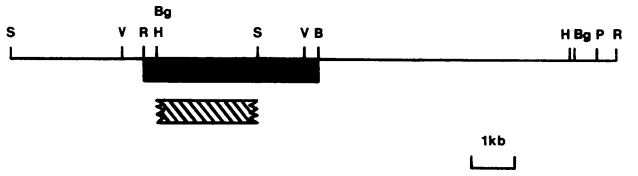

FIG. 2. *S. typhimurium* genomic DNA which hybridized to *E. coli umu* sequences. Restriction enzyme sites were deduced from Fig. 1 and similar experiments with other restriction endonucleases. Symbols: ■, *Bam*HI-*Eco*RI fragment cloned into pMH2532; ▨, minimum extent of hybridization with *E. coli umu* sequences. S, *Sal*I; V, *Eco*RV; R, *Eco*RI; Bg, *Bgl*II; H, *Hin*dIII; B, *Bam*HI; P, *Pst*I.

Hybridization of *E. coli umuD* and *umuC* probes was to a 2.2-kbp *Hin*dIII-*Sal*I fragment within the cloned sequence. This 2.2-kbp fragment from the cloned *S. typhimurium* DNA of clone 1 (Fig. 2) was used to reprobe the digested *S. typhimurium* DNA in Fig. 1a to confirm that the cloned material contained the same *S. typhimurium* sequence which hybridized to *E. coli umu*. This probe hybridized strongly to

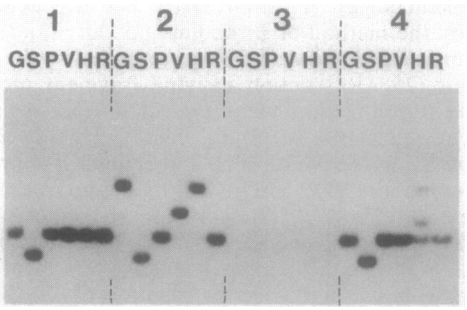

FIG. 3. Southern hybridization analysis of plasmid DNA from *S. typhimurium* library transformants 1, 2, 3, and 4 in *E. coli umuC122*::Tn*5*. The probe was the *E. coli umuC Bam*HI-*Sal*I fragment. G, *Bgl*II; S, *Sal*I; P, *Pst*I; V, *Eco*RV; H, *Hin*dIII; R, *Eco*RI.

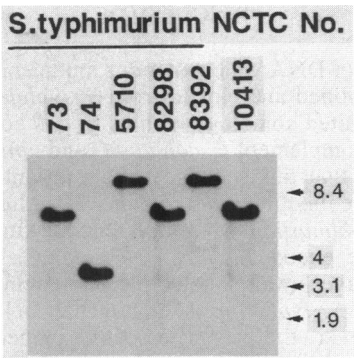

FIG. 4. Southern hybridization analysis of genomic DNA from natural isolates of *S. typhimurium* digested with *Hin*dIII and *Eco*RI and probed with the 2.2-kbp *Hin*dIII-*Sal*I fragment from pMH2532 encompassing *umuST*. Molecular sizes (kilobase pairs) are indicated on the right.

S. typhimurium genomic DNA, and the sequences that it hybridized to are identical to those detected with the *E. coli umuC* and *umuD* probes (Fig. 1c). Note that hybridization to the *S. typhimurium* genomic DNA was much stronger than with the *E. coli* probes shown in Fig. 1a and b. Hybridization to *E. coli umuC* DNA became visible only after extended exposure (data not shown). This experiment confirmed that the region of *S. typhimurium* genomic DNA, initially identified by its partial homology to *E. coli umuC*, is the material which was cloned and which gives functional complementation of *E. coli umuC122*::Tn*5* mutants. This hybridization information, together with the functional method of isolation, conclusively shows that a *umu*-like operon from *S. typhimurium* has been isolated. The plasmid carrying this sequence has been designated pMH2532.

In addition to hybridizing to genomic DNA from *S. typhimurium* LT2, the cloned material in pMH2532 also showed strong homology with genomic DNA from six independent stocks of wild-type *S. typhimurium* (Fig. 4). Thus, the sequence cloned in pMH2532 is a general feature of *S. typhimurium* and is not a specialized component of the LT2 subline. Note, however, that there are restriction site polymorphisms from stock to stock which are reminiscent of earlier observations made with *Escherichia* species (42).

Genetic characterization of pMH2532. The initial patch screening which produced pMH2532 showed that this plasmid was able to restore induced mutagenesis to an *E. coli umuC122*::Tn*5* mutant. A quantitative assay of induced mutability confirmed this conclusion and showed that pMH2532 provides a modest mutagenic response to UV radiation in a *umuC122*::Tn*5* strain (Fig. 5b). This response is clearly distinct from that of the nonmutable *umuC122*::Tn*5* parent but does not represent an increase in mutability to the level seen in the same strain carrying the *E. coli umuDC* plasmid, pSE117. pMH2532 similarly complemented the mutational deficiency of a *umuC36* mutant (data not shown). Both pMH2532 and pSE117 had only a small effect on the already-mutable *umu*$^+$ strain of *E. coli* (Fig. 5a). The enhanced mutagenesis conferred on *umuC122*::Tn*5* cells by pMH2532, like that conferred by pSE117, was dependent on the presence of a functional *recA* gene product, since *recA1 umuC122*::Tn*5* derivatives carrying either plasmid remained nonmutable by UV (Fig. 5d). Consistent with these results, both pMH2532 and pSE117 protected *umuC122*::Tn*5* cells from killing after UV irradiation, and protection conferred by pMH2532 was as great as that conferred by pSE117 (Fig. 6b). Neither plasmid appeared to enhance the survival of the resistant wild-type strain of *E. coli* (Fig. 6a) or the extremely sensitive *recA1 umuC122*::Tn*5* strain (Fig. 6c).

Previous studies have suggested that *S. typhimurium* does not carry a *umuD* activity (15). Therefore, complementation tests of *E. coli umuD* mutants were done in a *uvrA* genetic background to enhance the sensitivity with which *his-4* revertants could be detected. In *uvrA umuD44* mutants of *E. coli*, pMH2532 caused a small increase in the number of His$^+$ mutants detected after UV irradiation (Table 2). Note that the increase in mutagenesis was small in terms of the numbers of mutants per plate and that mutagenesis again

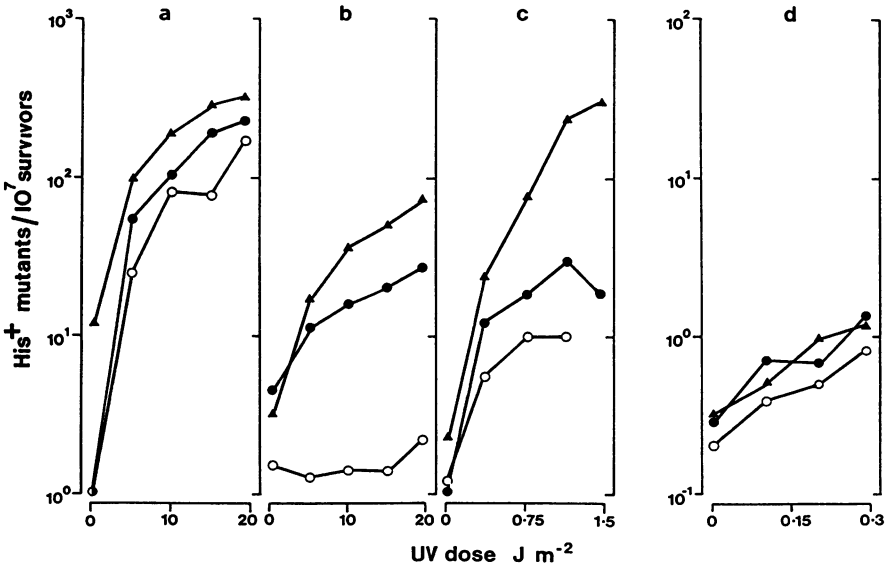

FIG. 5. Effect of pMH2532 on the frequency of His$^+$ reversion induced by UV irradiation in AB1157 *umu*$^+$ (a), AB1157 *umuC122*::Tn*5* (b), *S. typhimurium* TA2659 (c), and AB1157 *umuC122*::Tn*5 recA1* (d). Symbols: ○, no plasmid; ▲, pSE117; ●, pMH2532.

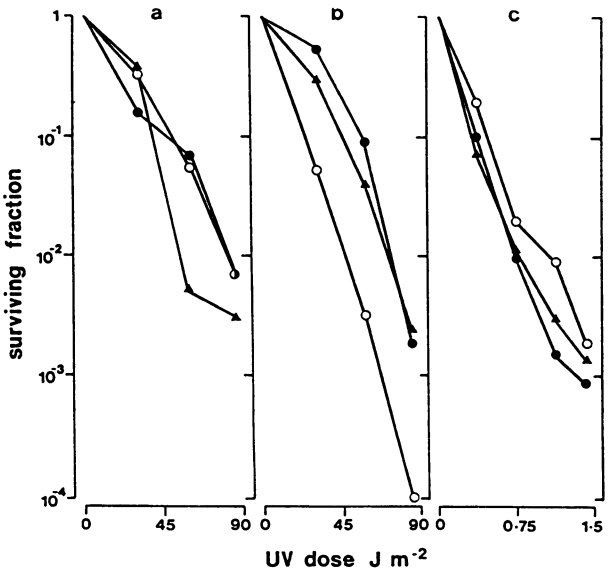

FIG. 6. Effect of pMH2532 on the survival after UV irradiation of AB1157 *umu*$^+$ (a), AB1157 *umuC122*::Tn*5* (b), and AB1157 *umuC122*::Tn*5* *recA1* (c). Symbols are as in Fig. 5.

was not restored to the levels observed with pSE117. It did, however, increase mutant numbers approximately sixfold above the spontaneous mutation frequency and above the mutation frequency of the non-plasmid-bearing parental strain. Similar experiments with an isogenic *umuD77* strain did not give this response, though such results may reflect differences in the nature of the point mutations in *umuD* being used for the study. Survival data were not gathered from *uvr* strains, since previous studies have shown that the *uvr* background can affect some of the protective effects of other plasmids carrying mutagenic repair genes (4).

To determine whether pMH2532 could also enhance mutagenesis in *S. typhimurium* like pSE117 (15), both plasmids were transformed into strain TA2659, which carries a UV-revertable *his* mutation and a *uvrB* deletion to enhance mutagenesis. pMH2532 increased the mutability of TA2659 to a level above that of the parent strain but not to the same extent as pSE117 (Fig. 6c). Note that this experiment also shows the weak mutability of the non-plasmid-bearing strain of *S. typhimurium*. Thus, pMH2532 encodes a function which can enhance mutagenesis of *E. coli umuC* and, to a much lesser extent, *E. coli umuD* and *S. typhimurium*.

TABLE 2. Effect of pMH2532 on UV-induced reversion to His$^+$ in *E. coli umuD44 uvrA*

UV dose (J m^{-2})	*E. coli umuD44 uvrA* plasmid	No. of viable cells/ plate (10^6)	No. of mutants/ plate	No. of induced mutants/10^7 survivor
0	None	20	4.5	0
	pMH2532	30	8	0
	pSE117	35	15.5	0
0.6	None	4.5	3.5	0
	pMH2532	6.8	20	17.8
	pSE117	2.6	150.5	529
0.9	None	0.1	0.5	0
	pMH2532	3.25	29.5	66
	pSE117	1.5	126.5	790

DISCUSSION

A segment of DNA which encodes mutagenic DNA repair has been identified and cloned from *S. typhimurium* on the basis of its limited homology with its *E. coli* counterpart and its ability to complement *E. coli umuC* and *umuD* mutations. It is possible that this sequence offers an entirely different and alternative function to *E. coli umuDC*. Alternatively, the cloned *S. typhimurium* DNA may encode functions analogous to those encoded by *E. coli umuDC*. Resolution of these alternatives awaits nucleotide sequencing and identification of the *S. typhimurium* mutagenic DNA repair protein(s). However, the properties of the cloned *S. typhimurium* DNA are like those of *E. coli umuDC* in several respects, and for this reason the cloned DNA has been provisionally designated *umuST*.

Evidence that *umuST* has *umuC*-like properties includes the following. First, *umuST* has positive hybridization with *E. coli umuC* sequences. Second, *umuST* is able to complement at least two different *umuC* mutations to restore both radioresistance and induced mutability. Third, *umuST* required a functional *recA* gene to induce mutagenesis. This means that *umuST* is like *umuDC* in needing SOS induction to be active.

umuST is also like *umuD*, though the *S. typhimurium* gene is much less active than its *E. coli* counterpart. Hybridization of *umuD* with *S. typhimurium* DNA was weak. Similarly, complementation of *umuD44* mutants by *umuST* was much less effective than complementation of *umuC* mutations.

A previous report of enhancement of *S. typhimurium* mutability by a cloned multicopy *umuD* gene was presented as evidence for the absence of an endogenous *umuD*-like gene (15). However, we have noted that multicopy *umuD* plasmids also enhance the mutability of wild-type *E. coli*, in which an endogenous *umuD* sequence is present (S. G. Sedgwick, unpublished observation). Thus, the effect of multicopy *umuD* genes may be to augment some *umuD* activity which is present but rate limiting. This might be especially the case with *S. typhimurium*, in which *umuD* activity appears particularly weak. It should be noted that all of the other three mutagenic DNA repair operons which have been structurally characterized have similar structures, with the larger *umuC/mucB/impB* gene preceded by a smaller *umuD/mucA/impA* gene (17, 34, 50).

Prior to these experiments there has been some conflict as to whether *S. typhimurium* had mutagenic DNA repair genes at all. Nonmutability of *S. typhimurium* in some assays was taken as evidence for the lack of mutagenic DNA repair (40, 45). However, in other cases increased yields of mutants were detected (15, 23, 31, 32). It is our experience that mutation fixation takes longer in *S. typhimurium* than in *E. coli* (unpublished observations). Thus, differences in expression times may have caused some of the differences in mutagenesis reported earlier. Results of a previous study which failed to detect DNA hybridization of *umuDC* with *S. typhimurium* (40) under more stringent conditions than those used here can now be reinterpreted as showing that *umu*-like sequences appear to have diversified much more than the other two repair genes, *recA* and *ada*, used in that study.

Our results show that *S. typhimurium* has an active *umuC*-like gene and an inefficient *umuD*-like sequence. It is possible that the reduced mutability of *S. typhimurium* strains with respect to *E. coli* is a direct result of this inefficient *umuD*-type gene. The actual extent of the diversification of *umuST* compared with that of *E. coli umuDC*

will be soon revealed by nucleotide sequencing, which is now under way. A comparison of conserved and variable features of the two mutagenic repair operons should point to structural features which relate to both their similarities and their differences in biological activities.

Upon completion of this work we learned that Smith and Eisenstadt had also detected *umuDC*-like DNA in the genome of *S. typhimurium* (47). Their report also suggests that *umuD*-like activity in *S. typhimurium* is weak and might limit the induced mutability of this bacterium.

ACKNOWLEDGMENTS

We thank M. Blanco, B. Bridges, L. Bullas, and G. Walker for their gifts of strains and plasmids and helpful advice. We are grateful to Geoffrey Banks and Kevin Hiom for stimulating discussions and advice and to Helen Crowne for technical assistance.

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