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Identification of a *umuDC* Locus in *Salmonella typhimurium* LT2

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The *umuDC* operon of *Escherichia coli* is required for efficient mutagenesis by UV light and many other DNA-damaging agents. The existence of a *umuDC* analog in *Salmonella typhimurium* has been questioned. With DNA probes to the *E. coli umuD* and *umuC* genes, we detected, by Southern blot hybridization, sequences similar to both of these genes in *S. typhimurium* LT2. We also confirmed that the presence of cloned *E. coli umuD* enhances the UV mutability and resistance of *S. typhimurium*. Our data strongly suggest that *S. typhimurium* contains a functional *umuDC* operon.

Salmonella typhimurium is widely used in the Ames test and related bioassays to identify mutagenic agents and potential human carcinogens in the environment (1, 22). The success of the Ames test in detecting the mutagenicity of a wide variety of DNA-damaging agents is, in large part, caused by the use of a mutation-enhancing plasmid, pKM101, which was introduced into the Ames tester strains (22). The necessity of this plasmid for efficient mutagenesis appears to be a consequence of a less-than-optimal mutability phenotype in *S. typhimurium* (25, 29, 39, 44).

In *Escherichia coli*, efficient mutagenesis by UV and many other DNA-damaging agents requires the chromosomal *umuDC* operon (14, 40). The *umuDC* operon is part of the *E. coli* SOS regulon, which consists of at least 16 different genes dispersed throughout the chromosome (33, 44). Transcription of the SOS regulon is controlled by LexA and RecA. After DNA damage or other treatments that block DNA replication, RecA becomes activated to a state that facilitates the autoproteolytic cleavage of the SOS repressor protein, LexA (18, 19). Activation of RecA leads, within minutes, to the depletion of LexA and the consequent derepression of SOS loci, including *umuDC* (17). In addition, UmuD is posttranslationally modified by RecA-mediated autoproteolysis to yield an active carboxy-terminal product (6, 26, 37).

Induced levels of both UmuD and UmuC, proteins of approximately 15 and 47 kilodaltons, respectively (12, 38), are needed for maximal SOS mutagenesis; mutations that inactivate either gene reduce the UV mutability of *E. coli* by more than 100-fold (14). Inactivation of *umuDC* also modestly increases the UV sensitivity of *E. coli* (14). UmuC and activated UmuD are both necessary (12), but may not be sufficient, for SOS-dependent mutagenesis (26). In addition to inactivating LexA and activating UmuD, a third function of RecA may also be required (26). Furthermore, recent studies suggest that two heat shock genes, *groEL* and *groES*, may also be involved in this process (C. Connelly and G. Walker, personal communication). The precise role of *umuDC* in mutagenesis remains unknown. It has been hypothesized that *umuDC* may not be directly involved in the misincorporation step but rather may function to enhance the ability of a stalled polymerase to replicate past blocking DNA lesions (4).

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UV mutability is not a common phenotype of bacteria in general or of members of the *Enterobacteriaceae* family in particular (44). Many plasmids, however, have been shown to carry genes that enhance mutagenesis and increase the UV resistance of their host (24, 34). These plasmids, which may encode *umu*-like functions, have been identified among at least 10 different plasmid incompatability groups from at least six different genera (for a review, see reference 41). Two analogs of *umuDC*, *mucAB* (31) and *impAB* (9), which were identified on the naturally occurring plasmids R46 and TP110, respectively, have been extensively studied. *mucAB* and *impAB* are regulated by RecA and LexA, share 35 to 50% DNA sequence similarity with *umuDC*, encode proteins of similar size, and restore mutability to *E. coli umuDC* mutants (9, 31, 32, 41). Complementation via exchange of the component proteins does not occur between UmuDC and MucAB, which indicates that each protein functions only with its cognate partner (31).

Although *umu*-like sequences are present on many plasmids, they have been detected either by functional criteria (2, 13) or by hybridization (35, 36) in only a few bacterial species. In fact, Sedgwick and Goodwin (35) have claimed that there is a correlation between the failure to detect *umuDC*-like sequences via hybridization in eight enterobacterial species, including *S. typhimurium*, and the failure to induce mutations in those species with UV irradiation. Other evidence also suggests that *S. typhimurium* may be deficient in *umuDC* function. In addition to the reported low mutability of this bacterium (25, 39, 44), it exhibits a poor ability to perform Weigle reactivation of UV-irradiated P22 bacteriophage (43). Weigle reactivation, the inducible, cell-mediated repair of UV-damaged phage, is an SOS-mediated process in *E. coli* that is highly dependent on *umuDC* (14). *E. coli umuDC* or its analog *mucAB* significantly enhances Weigle reactivation in *S. typhimurium* (44), which further suggests that this bacterium is deficient in *umuDC* activity. Yet, UV mutagenesis in *S. typhimurium* in the absence of pKM101 or other mutability-enhancing plasmids has been reported (13, 29, 30). Furthermore, UV mutability in *S. typhimurium* appears to be an inducible process (29), as it is in *E. coli*.

We have reexamined the question of whether *S. typhimurium* has sequences that are similar to *umuDC* from *E. coli*. Here we present molecular evidence that *S. typhimurium* does contain sequences similar to both *umuC* and *umuD*. We also present evidence that suggests that *S. typhimurium* contains a functional *umuC* gene that is able to interact with *E. coli umuD* provided on a high-copy-number plasmid.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Source or reference
S. typhimurium		
PM155	*leuD21*	30
CO109	*hisG46 zee*::Tn*10* derivative of PM155	C. Orrego
LB5000	$r^- m^+$	5
E. coli		
P90C	K-12, $umuD^+C^+$	7
HB101	*recA13* $r^- m^-$	3
Plasmids		
pGW1700	$mucA^+B^+$	32
pSE117	$umuD^+C^+$	21
pGW2020	$umuD^+C$	26
pMS2021	*umuD*Δ*C*	Derived from pGW2020 This work

While this work was in progress, Herrera et al. (13) reported that a different *umuD* construct enhanced the mutability of *S. typhimurium* Δ*uvrB*. The results presented here strongly suggest that *S. typhimurium* contains a functional *umuDC* operon.

MATERIALS AND METHODS

Bacterial strains and plasmids. The origins and relevant characteristics of bacterial strains and plasmids are summarized in Table 1. All plasmids are high-copy-number pBR322 derivatives. Plasmids were initially moved into *S. typhimurium* LB5000 by standard transformation techniques (16). Plasmids were moved between *S. typhimurium* strains by P22 transduction (8). All plasmid constructs were confirmed by restriction enzyme analysis.

Culture media and conditions. Bacteria were grown in NB medium (Nutrient Broth No. 2; Oxoid Ltd., London, England) at 37°C unless otherwise noted. Minimal medium was M9 medium with 0.2% glucose, as previously described (23), with 0.001% Casamino Acids (Difco Laboratories, Detroit, Mich.). For mutagenesis experiments with strain C0109, minimal medium was further supplemented with 1 μg of leucine per ml and 50 μg of histidine per ml. Survival determinations were made by using minimal plates with 50 μg of leucine per ml and 50 μg of histidine per ml. Medium for plasmid-bearing strains contained either 100 or 25 μg of ampicillin per ml for NB and minimal media, respectively.

Materials. Unless otherwise stated, all reagents were analytical grade. All enzymes were obtained from New England BioLabs, Inc., Beverly, Mass., and were used as recommended by the supplier.

DNA preparation. Genomic DNA was isolated from *E. coli* P90C and from *S. typhimurium* LT2 PM155 and was prepared essentially as previously described (10). Plasmid DNA was prepared by lysozyme-alkaline sodium dodecyl sulfate hydrolysis by standard methods (20).

Probe preparation. The probes used in this study are summarized in Fig. 1. *umuDC* and *umuD* probes were prepared by restriction of appropriate plasmids. Fragments were separated by gel electrophoresis and recovered from low-melting-point agarose. The *umuD*-specific plasmid (pMS2021) was constructed from pGW2020 by removing the *Nco*I (restriction site internal to *umuD*)-to-*Hin*dIII (restriction site downstream of *umuC*) fragment that contains all *umuC* sequences. This construction was confirmed via restriction analysis and further shown by Southern blot hybridization to lack *umuC* sequences. For Southern blot hybridizations, 80 to 120 ng of probe was labeled as previously described (20).

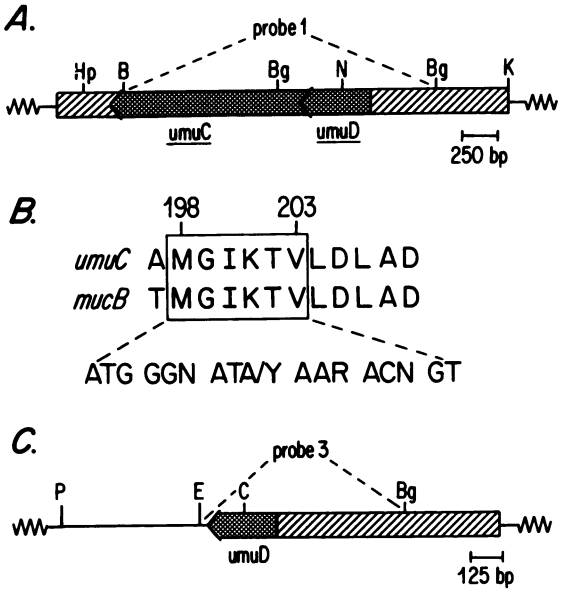

FIG. 1. *umuDC* probes used to detect sequences similar to *umuDC* in *S. typhimurium*. Probes were isolated as described in Materials and Methods. (A) Probe 1 was obtained from pSE117 by digestion with *Bgl*II and *Bam*HI. This probe spans both *umuD* and *umuC* and includes approximately 450 base pairs (bp) of flanking upstream sequence. (B) Probe 2 is a degenerate (96-fold) oligonucleotide mixture to the region that spans amino acid residues 198 to 203 of UmuC and MucB (30). (C) Probe 3 (described in Materials and Methods [Probe preparation]) spans approximately 250 base pairs of the amino-terminal portion of *umuD* and includes upstream flanking sequence. In panels A and C, vector sequences are identified by solid lines; cloned sequences are identified by boxes. □, *umuDC* sequences; ▨, flanking sequences. Abbreviations: N, *Nco*I; Bg, *Bgl*II; B, *Bam*HI; K, *Kpn*I; Hp, *Hpa*I; P, *Pvu*II; C, *Cla*I; E, *Eco*RI.

Southern blot hybridization. Samples of genomic or plasmid DNA were restricted and prepared for Southern blot hybridization by standard methodology (20). For Southern blot hybridizations with nick-translated fragments specifying *umuDC* and *umuD*, prehybridizations and hybridizations were done overnight at 55°C unless otherwise noted. Prehybridization solution contained 3× Denhardt solution, 0.1 M MOPS (morpholinepropanesulfonic acid)-free acid, 0.8 M NaCl, 0.1% sodium lauroyl sarkosine, and 250 μg of herring sperm DNA per ml. Hybridization solution was the same, except with 2× Denhardt solution and 100 μg of herring sperm DNA per ml. Radioactively labeled probes were used at 4×10^5 to 8×10^5 cpm per ml in each hybridization. Unless noted otherwise, final washes (twice for 15 min each time) were at the temperature of hybridization with 2× wash solution (2× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate] plus 0.1% sodium lauroyl sarkosine and 0.05% sodium pyrophosphate). Southern blot hybridizations with the oligonucleotide probes were performed essentially as previously described (R. A. Zeff and J. Geliebler, BRL Focus **9**:1–2, 1987).

UV mutagenesis and killing. Bacteria containing plasmids were grown overnight at 30°C in minimal medium supplemented for all auxotrophies and containing 25 μg of ampicillin per ml. The next day, cultures were diluted 100-fold into fresh minimal medium containing 25 μg of ampicillin per

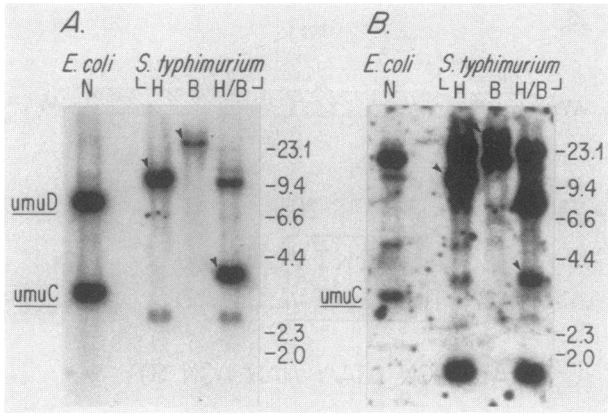

FIG. 2. *S. typhimurium* genomic DNA digests contain sequences similar to *E. coli umuC*. Each lane was loaded with approximately 1.5 μg of *E. coli* or *S. typhimurium* genomic DNA digested with the enzymes noted and electrophoresed through a 0.7% agarose gel. Panels are of sister gels run side by side: (A) *umuDC* probe; (B) degenerate *umuC* oligonucleotide probe (descriptions of both probes are the same as in the legend for Fig. 1A and B). Numbers at the right indicate molecular size standards in kilobases. The enzyme *Nco*I cuts *E. coli umuDC* internally, which generates a fragment that contains only a portion of *umuD* and a second fragment with the remaining *umuD* and *umuC* sequences. These fragments are labeled. Arrows indicate *S. typhimurium* fragments identified by both the *umuDC* full-length and *umuC* oligonucleotide probes. Abbreviations: N, *Nco*I; H, *Hin*dIII; B, *Bam*HI; H/B, *Hin*dIII and *Bam*HI double digest.

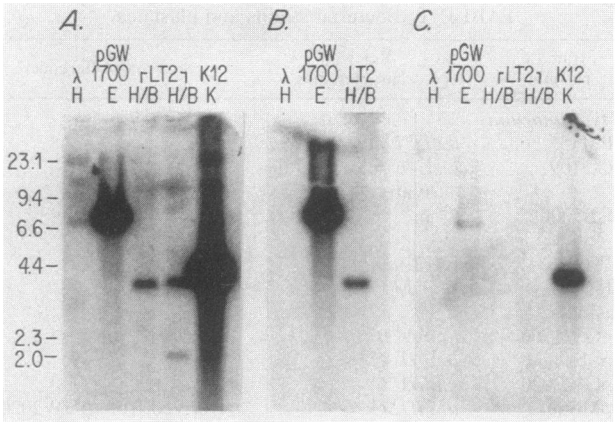

FIG. 3. Hybridization of *E. coli umuDC* to *S. typhimurium* genomic DNA and to *mucAB* under conditions of increasing stringency. pGW1700 plasmid DNA (bearing cloned *mucAB*) was linearized with *Eco*RI, and 250 ng was loaded to the second lane of each panel. *E. coli* and *S. typhimurium* genomic lanes contain approximately 1 μg of DNA each. An additional 2-min wash in 0.2× wash solution at the temperature of hybridization was performed. Hybridization and washes were at 51°C (A), 59°C (B), or 65°C (C; note loss of hybridization to *S. typhimurium* 3.7-kb fragment [lanes 3 and 4] and to *mucAB* containing pGW1700 [lane 2]). Numbers at the right are molecular size standards in kilobases. Abbreviations: H, *Hin*dIII; E, *Eco*RI; K, *Kpn*I; H/B, *Hin*dIII and *Bam*HI; K12, *E. coli* K-12 P90C; LT2, *S. typhimurium* LT2 PM155.

ml. At cell densities of approximately 2×10^8/ml, 4 ml of each culture was removed to large glass petri dishes and irradiated with constant agitation at the fluences noted. For each dose, portions were removed immediately, diluted into 0.85% NaCl, and plated for survivors on minimal plates. Revertants were scored on minimal plates supplemented as described above. Plates were incubated at 30°C. Mutants were scored after 3 days.

RESULTS

Identification of *S. typhimurium* sequences that hybridize to *E. coli umuDC*. DNA fragments spanning the entire *E. coli umuDC* operon, which includes approximately 400 base pairs of upstream flanking sequence (Fig. 1A), were used to probe electrophoretically separated restriction fragments of *S. typhimurium* LT2 genomic DNA. For comparison, *E. coli* genomic DNA digests were also probed. The autoradiographs shown in Fig. 2A demonstrate that, under conditions of moderate stringency, this probe hybridized to specific restriction fragments of *S. typhimurium* LT2 genomic DNA (lanes 3 through 5). As expected, the probe also hybridized to the *E. coli umuDC*-containing fragments (Fig. 2A, lane 1).

Under the conditions used in these experiments, the hybridization signal to *S. typhimurium* DNA was considerably weaker than that obtained to an equivalent quantity of *E. coli* genomic DNA. The autoradiographs shown in Fig. 3 illustrate that, as the stringency conditions of hybridization and washes were increased, hybridization of this probe to *E. coli* DNA remained strong but hybridization to both *S. typhimurium* DNA and *mucAB* DNA diminished substantially. As the DNA sequence similarity between *mucAB* and *umuDC* is only approximately 50%, these hybridization results are not unexpected and indicate that the *S. typhimurium* sequences identified share only partial similarity with *E. coli umuDC* sequences.

To verify that the *S. typhimurium* restriction fragments detected by the *E. coli umuDC* DNA probe belonged to a *umuDC*-like locus rather than to conserved regions in flanking sequences, we used a probe specific to *umuC*. This probe was a synthetic 17-base-pair oligonucleotide with a 96-fold degeneracy that corresponds to one of several regions where the deduced amino acid sequences are identical between *umuC* and *mucB* (Fig. 1B). The autoradiograph shown in Fig. 2B demonstrates that this degenerate *umuC* probe hybridized to the same restriction fragments of *S. typhimurium* genomic DNA detected by the *E. coli umuDC* probe. Additional restriction fragments from both *S. typhimurium* and *E. coli* were also detected with this degenerate oligonucleotide, which suggests that this amino acid sequence may be a conserved motif found in several proteins. A second oligonucleotide probe to a different region of conserved amino acid sequence between *umuC* and *mucB* (amino acid residues 50 through 55) (31) also hybridized to the same fragments detected by both the *umuDC* probe and the first oligonucleotide (data not shown).

A partial restriction map of the *S. typhimurium umuDC* region was constructed by using the *umuDC* probe. The operon appears to reside wholly within two contiguous *Hin*F fragments spanning 2.9 kilobases and also within a 3.7-kilobase *Bam*HI-*Hin*dIII fragment.

***S. typhimurium* contains sequences similar to *umuD*.** Our results suggest that *S. typhimurium* has a *umuC* gene. These observations together with the demonstrated UV mutability of *S. typhimurium* (13, 29, 30) led us to ask whether sequences similar to *umuD* are also present in this bacterium. To test whether *S. typhimurium* does, in fact, contain sequences similar to *E. coli umuD*, we probed *S. typhimurium* genomic digests with a fragment of a derivative of the *umuDC*-containing plasmid pGW2020, from which the entire *umuC* locus and flanking downstream sequences had been deleted (pMS2021; Fig. 1C). The same restriction fragments

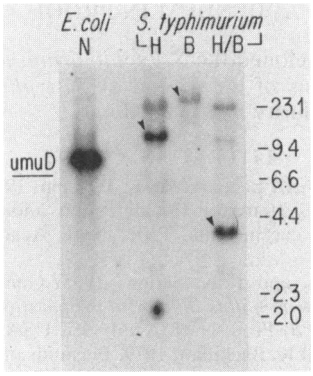

FIG. 4. *S. typhimurium* contains sequences that hybridize to *E. coli umuD*. Southern blot hybridizations were performed with probe 3 (described in the legend to Fig. 1C). The filter represents a third sister gel to those presented in Fig. 2. The position of the *E. coli umuD*-containing fragment is indicated. Lack of signal at the *E. coli umuC* fragment confirms the absence of *umuC* sequences in the probe preparation. Arrows indicate *S. typhimurium* fragments bearing sequences similar to *umuD*. These same fragments are also detected by a *umuC*-specific oligonucleotide (Fig. 3B). Numbers at the right are molecular size standards in kilobases. Abbreviations: H, *Hin*dIII; B, *Bam*HI; H/B, *Hin*dIII and *Bam*HI.

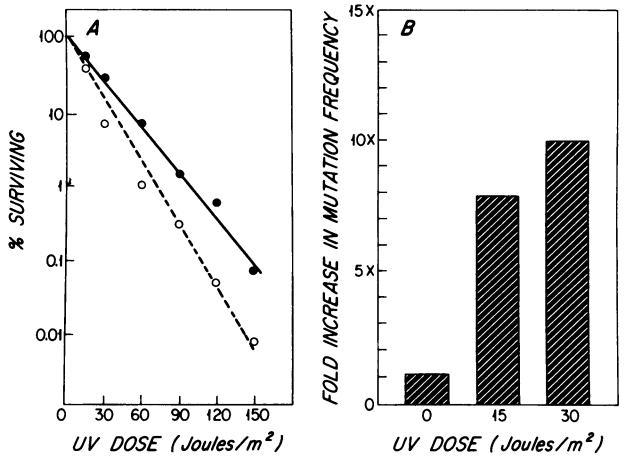

FIG. 5. UV-induced mutagenesis and cell killing of *S. typhimurium* LT2 bearing plasmids pGW2020 (*umuD*$^+$) and pMS2021 (*umuD* mutant). Strain C0109 was grown and irradiated as described in Materials and Methods. (A) Irradiated bacteria bearing *umuD* (●) and control (○) plasmids were plated for surviving fractions. Each point represents the average of three plates from each of two independent experiments. (B) Reversion frequencies of *leuD21* were determined by spreading 0.1 ml of each irradiated culture of pGW2020- and pMS2021-bearing strains on leucine-limiting plates. Plates were incubated at 30°C and scored after 3 days. Frequencies were adjusted for spontaneous mutations and were determined on the basis of the average of three plates from each of two independent experiments. The numbers of *leuD21* revertants per plate at each fluence, with bacteria carrying the *umuD* mutant plasmid, were (i) 3.5 with no irradiation, (ii) 4.7 with 15 J/m^2, and (iii) 4 with 30 J/m^2.

that were detected by both the *umuDC* probe and *umuC*-specific oligonucleotide probes also hybridize to this *umuD* probe (Fig. 4). Thus, we conclude that *S. typhimurium* contains sequences that are similar to *E. coli umuD* as well as to *umuC*. As the *umuD*- and *umuC*-like sequences reside on the same 3.7-kilobase *Bam*HI-*Hin*dIII fragment, these loci are not widely separated and are likely to be contiguous as are their counterparts in *E. coli*.

***S. typhimurium* contains a functional *umuC* gene.** We reasoned that the *E. coli* and *S. typhimurium umuDC* alleles might be more closely related to one another than to the plasmid-borne *mucAB* locus, since the latter may have been subject to different selective pressures during the course of evolution. Therefore, we asked whether *E. coli umuD* would enhance *S. typhimurium* mutability. In fact, when we introduced a high-copy-number plasmid that carries the *E. coli umuD* gene into *S. typhimurium*, we observed that, in comparison with a control plasmid deleted for *umuD*, both UV resistance and mutability were enhanced. The increase in resistance to the lethal effects of UV was modest, approximately 30%, but reproducible (Fig. 5A). The frequency of UV-induced reversion of *leuD21* was stimulated by *E. coli umuD* up to 10-fold (Fig. 5B). Under the conditions used in these experiments, UV mutability was dependent upon the presence of *E. coli umuD*. However, it must be noted that the UV mutability of *S. typhimurium* is known to vary with experimental conditions. For example, UV irradiation of *S. typhimurium* spread on plates induces a substantial level of mutagenesis (11). *E. coli umuD* also enhances the UV mutability of *leuD21* three- to fivefold under these latter conditions (data not shown).

DISCUSSION

We have shown that *S. typhimurium* contains sequences similar to both *E. coli umuC* and *umuD*. The same restriction fragments from digests of *S. typhimurium* genomic DNA were detected with four different DNA probes: the *umuDC* operon with upstream flanking DNA, two different degenerate oligonucleotides specific to regions with identical amino acid sequences shared between UumC and MucB, and a *umuD* probe. The possibility that the hybridization we observed was caused by conserved sequences in the regions flanking *umuDC* is unlikely, since two oligonucleotide probes to two different regions of *umuC* hybridized to the same restriction fragments as did the *umuDC* and *umuD* probes. These observations differ from those of Sedgwick and Goodwin (35), who did not detect *umuDC* sequences in *S. typhimurium* genomic DNA with dot blots. Their results may have been a consequence of hybridization and wash conditions that were too stringent to detect a low degree of similarity between the *E. coli* and *S. typhimurium* DNA sequences in the *umuDC* locus. Supporting this possibility, we found that, under conditions of higher stringency, our *umuDC* probe bound poorly to both *mucAB* sequences and *S. typhimurium* DNA. Thus, the conclusion that *umuDC*-like sequences are not widely distributed among enterobacterial species bears reevaluation. A wider distribution of *umu*-like sequences is supported by the apparent presence of a *mucB* analog in *Haemophilus influenzae* (2), an organism reported to be nonmutable by UV (27).

Our results are in agreement with the findings of Herrara et al. (13) that the *E. coli umuD* locus on a high-copy-number plasmid enhances the UV mutability and resistance of *S. typhimurium*. Herrara et al. (13) reported that cloned *E. coli umuDC*, which carries a Tn*1000* insertion in *umuC*, significantly enhanced the reversion frequency of *hisG428* in *S. typhimurium* Δ*uvrB*. Our results with a different *umuDC* construct which contained a functional *umuD* and an inactive truncated *umuC* extend these observations. We have shown that *E. coli umuD* enhances the UV-induced mutagenesis of an additional locus, *leuD21*, in *uvrB*$^+$ *S. typhimurium* by up to a factor of 10 and increases the UV resistance

of this strain by approximately 30%. In *E. coli*, *umuD* alone is not sufficient for SOS mutagenesis; both *umuD* and *umuC* are required (44). Therefore, the ability of *umuD* to enhance the UV mutability and resistance of *S. typhimurium* suggests that this bacterium contains a functional *umuC* analog. Although one cannot rule out the formal possibility that *E. coli umuD* enhances UV mutability in *S. typhimurium* by a process that does not require *umuC*, we think it extremely unlikely.

From these observations, we favor the hypothesis that *umuD* activity is a limiting component for UV mutagenesis in *S. typhimurium*. We do not believe, however, that this deficiency is complete because (i) *S. typhimurium* is mutable by UV under certain conditions (13, 29, 30), (ii) UV mutagenesis in *S. typhimurium* is inducible (29), and (iii) our results indicate that sequences similar to *umuD* are indeed present in this bacterium. These observations suggest to us that a *umuD* analog is also present and functional to a limited degree in *S. typhimurium*. Confirmation of this hypothesis awaits the cloning and characterization of the putative *S. typhimurium umuD* analog or isolation of mutants of this gene.

The ability of *E. coli umuD* to enhance the UV mutability and resistance of *S. typhimurium* suggests that the *umuC* allele in this bacterium is structurally more closely related to its *E. coli* counterpart than is the plasmid analog *mucB*. If the ancestral *umuDC* locus originated prior to the evolutionary split between *E. coli* and *S. typhimurium*, this finding suggests that, despite over 10^8 years of evolutionary divergence (28), there has been a significant degree of conservation of structure and function of at least UmuC. A second possibility for the origins of these alleles is that they may have been independently and more recently introduced into both organisms from related extrachromosomal sequences. Several investigators have proposed that *umuDC* and *mucAB* may have originated as part of a transposable element (2, 9, 15, 35). Sedgwick et al. (36) have recently shown that the *umuDC* locus in *E. coli* has features that are suggestive of a transposable element. The *muc* genes of pKM101 are also bounded by inverted sequences that are suggestive of a transposon (15), and other *umuDC* plasmid analogs are found on multiple-transposon derived plasmids (42). The isolation and characterization of the *S. typhimurium umuDC* analog at the nucleotide sequence level will allow one to further address evolutionary questions that concern the origins and functions of this locus in enterobacteria.

ACKNOWLEDGMENTS

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ADDENDUM IN PROOF

We have now cloned the *S. typhimurium umuDC* analog. Sequence analysis of the 3′ end of *S. typhimurium umuC* confirms its similarity to *E. coli umuC*.

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