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Mutagenic DNA Repair in Enterobacteria

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Sixteen species of enterobacteria have been screened for mutagenic DNA repair activity. In *Escherichia coli*, mutagenic DNA repair is encoded by the *umuDC* operon. Synthesis of UmuD and UmuC proteins is induced as part of the SOS response to DNA damage, and after induction, the UmuD protein undergoes an autocatalytic cleavage to produce the carboxy-terminal UmuD′ fragment needed for induced mutagenesis. The presence of a similar system in other species was examined by using a combined approach of inducible-mutagenesis assays, cross-reactivity to *E. coli* UmuD and UmuD′ antibodies to test for induction and cleavage of UmuD-like proteins, and hybridization with *E. coli* and *Salmonella typhimurium umu* DNA probes to map *umu*-like genes. The results indicate a more widespread distribution of mutagenic DNA repair in other species than was previously thought. They also show that *umu* loci can be more complex in other species than in *E. coli*. Differences in UV-induced mutability of more than 200-fold were seen between different species of enteric bacteria and even between multiple natural isolates of *E. coli*, and yet some of the species which display a poorly mutable phenotype still have *umu*-like genes and proteins. It is suggested that *umuDC* genes can be curtailed in their mutagenic activities but that they may still participate in some other, unknown process which provides the continued stimulus for their retention.

Mutagenesis induced by UV radiation and many chemical agents in *Escherichia coli* occurs through the action of a mutagenic DNA repair system acting on DNA lesions. When this system is absent, as in *umuDC* mutants, no mutagenic processing occurs and there is no induced mutagenesis (16, 46). *umuDC* is a dicistronic operon which apparently encodes structural genes for mutagenic repair (8, 17, 32, 40). Expression of *umuDC* is controlled by the SOS response to DNA damage. In an uninduced cell, LexA, the repressor of SOS genes, maintains synthesis of UmuD and UmuC at low basal levels (2, 51). After exposure to DNA replication-inhibiting agents such as UV light, the autocatalytic cleavage of LexA results in the inactivation of repressor function and leads to derepression of SOS-controlled genes. In vitro this reaction occurs at alkaline pH or, at more physiological conditions, after interaction with a ternary complex of RecA protein, single-stranded DNA, and ATP (19, 42, 43). Elevated levels of the UmuDC proteins are not sufficient to promote mutagenesis. UmuD must be posttranslationally modified to an active form, UmuD′, by autocatalytic cleavage, triggered by the same complex of RecA, single-stranded DNA, and ATP (5, 29, 39).

In addition to the chromosomal *umuDC* operon of *E. coli*, mutagenic DNA repair activity can be provided by analogous genes carried by a wide variety of conjugative plasmids from several different incompatibility groups (for a review, see reference 47). The best characterized of these genes are *mucAB* and *impCAB*, which share with *umuDC* similar operon sizes, organization, gene products, and SOS regulation (9, 20, 32).

SOS regulatory networks appear to be widespread in prokaryotes. Conservation of RecA protein, required for positive regulation, is considerable. Indeed, the facility of cloning heterologous *recA* genes by complementation of *E. coli recA* mutants emphasizes this conservation (for reviews, see references 26, 33, and 34). Similarly, there is considerable evidence for the widespread presence of LexA-like activities and damage-inducible gene expression (21, 26, 36).

Set against the solid background of conservation of the SOS response, the evidence for conservation of mutagenic DNA repair is much less certain. The levels of induced mutagenesis in *E. coli* are much higher than those found in many other species (13, 18, 22, 28, 35, 41). It was originally thought that the reduced mutability was due to the absence of *umu*-like genes or proteins. For instance, the idea that *Salmonella typhimurium* was *umu* defective gained strength with the demonstration that conjugation of the *E. coli umuDC* region restored mutagenesis (41). Similarly, introduction of *E. coli umuD* plasmids enhanced mutability, suggesting that the limitation of mutagenesis was the result of *umuD* (11, 44). Nevertheless, mutagenesis relying on SOS induction was demonstrated in *S. typhimurium* (31), and it was ultimately shown that *S. typhimurium* does indeed have a chromosomal *umuDC* operon despite all the previous indications to the contrary (44, 45, 48, 49). In fact, *S. typhimurium* LT2 contains two *umuDC*-like operons; the second, designated *samAB*, is encoded by the cryptic LT2 plasmid (30).

The finding that *S. typhimurium* was poorly mutable yet contained at least two *umu*-like operons suggested that *umuDC*-like sequences or proteins may be present in a variety of enteric bacteria once thought to be devoid of these functions. The aim of this paper is to confirm the existence of mutagenic DNA repair responses in a variety of enteric bacteria and to establish the widespread occurrence of *umu*-like genes and proteins. To achieve this goal, we have used a combined approach of inducible mutagenesis assays, cross-reactivity with *E. coli* UmuD and UmuD′ antibodies, and hybridization with *E. coli* and *S. typhimurium umu* DNA sequences.

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TABLE 1. Bacteria

Species	Stock	Source or reference
E. coli	AB1157	14
	DE1500	51
	MH31 to 43	38
E. dispar	NCTC 4168, 4450, 7921, 8006	National Collection of Type Cultures
E. alkalescens	NCTC 1601, 5183, 7925, 7928, 9775, 10764	National Collection of Type Cultures
E. aurescens	NCTC 10855	National Collection of Type Cultures
E. adecarboxylata	NCTC 10600	National Collection of Type Cultures
E. blattae	NCTC 10965	National Collection of Type Cultures
E. fergusonii	CL796/77	National Collection of Type Cultures
E. vulneris	CL777/77	National Collection of Type Cultures
E. hermanii	CL157/82	National Collection of Type Cultures
S. sonnei	NCTC 10342	National Collection of Type Cultures
S. boydii	NCTC 9355	National Collection of Type Cultures
K. aerogenes	NCTC 418	National Collection of Type Cultures
S. typhimurium	LT2	J. Bulas
	TA1977	1
P. mirabilis	PG1300	7
P. rettgeri	MH10	35
C. intermedius	MH23	35

MATERIALS AND METHODS

Bacterial stocks and culture. Bacterial species and stocks used are described in Table 1. They were cultured on L agar or broth at 37°C (23).

Plasmids. pMH2532 encodes the *S. typhimurium umuDC* operon in a 3.9-kbp *Bam*HI-*Eco*RI fragment (49). pSE117 and pLM207 encode *E. coli umuDC* (5, 24). pSK100 consists of a 19-kbp *Eco*RI fragment encompassing *E. coli umuDC* in the low-copy-number vector pMF3 (40).

UV-induced mutagenesis. UV mutability was assayed by the induction of resistance to rifampin (100 μg/ml) by using the triple-overlay technique as previously described (35).

Enzymes and biochemicals. Restriction enzymes were from Bethesda Research Laboratories. [α-^{32}P]dTTP phate was from New England Nuclear Inc. Rifampin was from Sigma.

Southern hybridization. DNA extraction and Southern hybridization were conducted as previously described (38). The *E. coli umuD* probe was a 0.9-kbp *Bgl*II-*Hin*cII fragment of pLM207. The *umuC* probe was a 1.2-kbp *Bam*HI-*Sal*I fragment from pSE117. A 19-kbp *Eco*RI fragment spanning the *E. coli umuDC* region was prepared from pSK100. The *S. typhimurium umuDC* probe was the 3.9-kbp *Bam*HI-*Eco*RI fragment of pMH2532. Hybridization was conducted at 65°C in 0.75 M NaCl–75 mM trisodium citrate–50 mM NaH_2PO_4–5 mM EDTA (5× SSCPE)–0.2% sodium dodecyl sulfate–75 μg of heat-denatured salmon sperm DNA per ml with two 10-min washes at 65°C in 5× SSCPE–0.2% sodium dodecyl sulfate.

Western blotting (immunoblotting). Bacterial whole-cell extracts were obtained as described elsewhere (51). Where indicated, cells were treated with 1 μg of mitomycin C per ml for 2 h prior to harvesting to induce any SOS-like response.

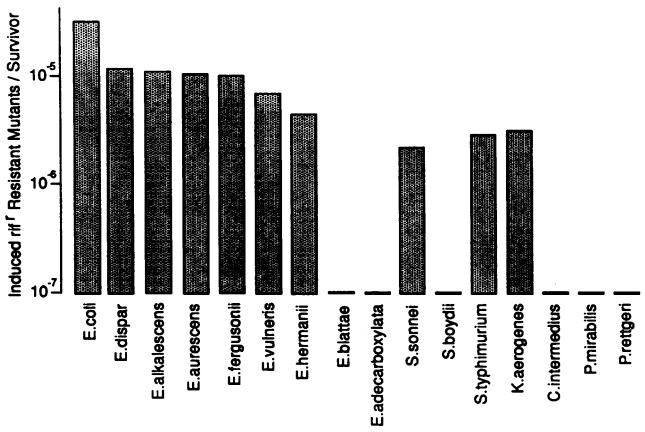

FIG. 1. Variation in induced mutagenesis in enterobacteria. Induced mutagenesis to rifampin resistance after 6 J of UV per m^2 is shown for the species indicated.

Proteins were transferred to nitrocellulose membranes by standard techniques (23). Membranes were probed with a 1:10,000 dilution of UmuD and UmuD′ antibody (a kind gift from H. Echols, University of California, Berkeley). Proteins were visualized with the Western Light chemiluminescence detection assay (Tropix, Bedford, Mass.).

RESULTS

UV-induced mutability of enterobacteria. Induced mutagenesis to rifampin resistance was used as an indicator of *umuDC* activity in the range of enterobacteria under study. UV dose-response experiments were performed two or more times with four or more UV doses over the range of 0 to 10 J/m^2 for each species. For ease of comparison of results from multiple experiments, results obtained after a single dose are presented (Fig. 1). The single dose points presented were obtained before plateau levels in the mutagenic response to UV were reached. Induced mutagenesis was detected in *E. coli*, *Escherichia dispar*, and *Escherichia alkalescens*. However, further examination of multiple isolates of these species from the wild revealed that there can be large differences in their UV-induced mutabilities and UV sensitivities. For example, in *E. coli* isolates there was a striking range of mutability and sensitivity, with the laboratory *E. coli* stock, AB1157, being grouped with the most mutable and resistant isolates (Fig. 2). In these stocks, there were also differences in the basal levels of mutants in the unirradiated samples; however, in no case did abnormally high spontaneous frequencies cause deceptive nonmutable assignments when UV irradiation failed to increase mutagenesis further. A similar range of UV-induced mutability was seen in a collection of *E. coli* natural isolates from the Murray collection (15) of pre-antibiotic era bacteria (data not shown).

Within the genus *Escherichia* itself there were also marked differences in mutability. Whereas induced mutagenesis to rifampin resistance was poor or absent in *E. blattae* and *E. adecarboxylata*, there were increasingly stronger responses with *E. hermanii*, *E. vulneris*, *E. fergusonii*, and *E. aurescens*. Other enterobacteria also reflected this range of mutability. Compared with levels in *E. coli*, lower levels of mutagenesis were found in *Klebsiella aerogenes*, *Shigella sonnei*, and *S. typhimurium*. Induced mutagenesis was not detectable in *Proteus mirabilis*, *Proteus rettgeri*, or *Shigella*

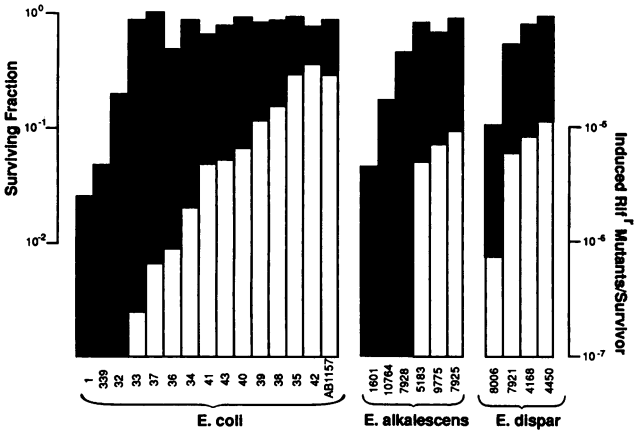

FIG. 2. Variation in intraspecies UV-induced mutagenesis and UV resistance of multiple natural isolates of *E. coli*, *E. alkalescens*, and *E. dispar*. The surviving fraction (solid histogram) and the induced frequency of mutagenesis to rifampin resistance (open histogram) are shown after a UV dose of 6 J/m^2.

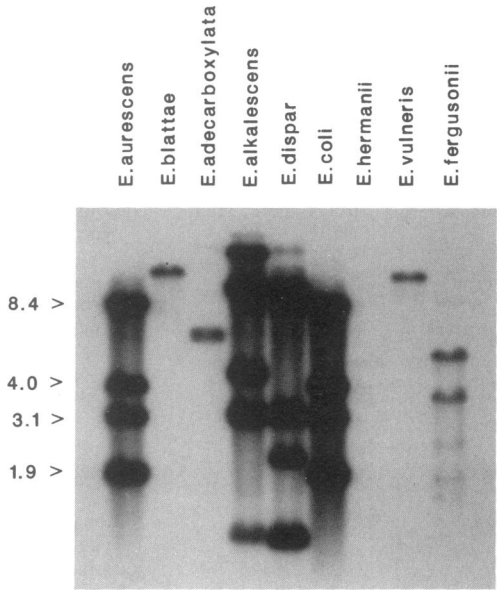

FIG. 3. Identification of *umu*-like DNA in *Escherichia* species. Genomic DNA from the species indicated was digested with *Hin*dIII and *Eco*RI and subjected to Southern hybridization with a 19-kb *Eco*RI probe containing the *umuDC* region of *E. coli*.

boydii. In summary, these mutagenesis data show that differences in induced mutagenesis of more than 200-fold can be seen between different enterobacteria and even between multiple isolates of a single species.

***umu*-like DNA in enterobacteria.** The presence of *umu*-like sequences in enterobacteria was tested by Southern hybridization. Two approaches were used. In cases in which closely related organisms were examined, it was sufficient to use an *E. coli* probe to identify strongly hybridizing material. In cases in which the homology was weaker, *umu*-like DNA was defined by cohybridization between probes comprising *S. typhimurium umuDC* and nonoverlapping *umuC* and *umuD* probes from *E. coli*. Bands which were common to two or even three probes were judged to be *umu* specific and used to generate restriction site maps.

DNA from nine *Escherichia* species was examined for *umu*-like sequences by hybridization with a 19-kb *Eco*RI fragment from pSK100 encompassing *E. coli umuDC* and flanking DNA (Fig. 3). Approximately equally intense hybridization was found with DNA from *E. coli*, *E. dispar*, *E. alkalescens*, and *E. aurescens*. Hybridization was much poorer with *E. adecarboxylata* and *E. blattae*, becoming even weaker with *E. fergusonii* and *E. vulneris* and almost undetectable with *E. hermanii*. In *E. coli*, *E. dispar*, *E. alkalescens*, and *E. aurescens*, the strongly hybridizing DNA includes a common 3.1-kbp *Hin*dIII-*Eco*RI band which previous mapping studies have shown to encompass a common *umu* region (38) (Fig. 4).

Restriction maps of *umu*-like regions in DNA from the five poorly hybridizing *Escherichia* species were produced by hybridization of multiple pairwise restriction digests with *Pst*I, *Eco*RV, *Ava*II, *Bam*HI, and *Eco*RI probed sequentially with smaller *umu*-specific probes from both *E. coli* and *S. typhimurium* (Fig. 5). *E. fergusonii*, *E. hermanii*, and *E. blattae* were mapped on the basis of bands cohybridizing with at least two probes. *umu*-like sequences also appear to be present in *E. vulneris* according to the same criterion of cohybridization, but despite this positive indication of *umu*-like genes, it has proved impossible to generate a restriction map, possibly because the probes used are homologous with multiple noncontiguous target sequences.

A complex pattern of cohybridization between *S. typhimurium umuDC* and *E. coli umuD* and *umuC* probes in *E. adecarboxylata* generated overlapping maps wherein *umuD* hybridized to two closely linked sites, one of which is unique to the *E. coli umuD* probe and one of which is homologous to the *E. coli umuC* probe. The two sites are separated by a tract which is homologous to *umuC* but not to *umuD*. Thus, *E. adecarboxylata* does have *umu*-like sequences in its genome; in fact, it may have two similar, but not identical, *umu* regions.

Outside of the genus *Escherichia*, DNA from *S. sonnei* and *S. boydii* hybridized most efficiently. In fact, the restriction site map of *S. boydii* is very similar to that of *E. coli* except for the position of a *Bgl*II site (Fig. 4). The map for the *umu* region of *S. sonnei* differed only by the insertion of an additional 2-kbp *Bam*HI fragment in the region thought to encompass *umuC* (Fig. 4). The presence of *umu*-like sequences in *K. aerogenes* and their restriction site map was based on the presence of common bands of hybridization between *E. coli umuC* and *S. typhimurium* probes (Fig. 5).

Citrobacter intermedius DNA gave very complex patterns of hybridization, portions of which are shown in Fig. 6. Cohybridizing bands generated two noncontiguous maps (Fig. 5). These maps of *C. intermedius* were based on the hybridization bands with the *S. typhimurium umuDC* probe which were common with bands produced by either *E. coli umuD* or *umuC* probes. It is not known whether the remainder of the uniquely hybridizing bands still represent *umu* homology or are the result of spurious cross-hybridization to unrelated sequences. Hence the map of *umu*-like sequences in *C. intermedius* must be regarded as incomplete, even though it already appears to be more complex than those of many other species.

No hybridization could be detected with any *umu* probe against DNA from either *P. mirabilis* or *P. rettgeri*. For completeness, the same restriction sites in the *S. typhimurium umu* region are included in Fig. 5 (45, 48).

UmuD proteins in enterobacteria. In *E. coli*, the SOS

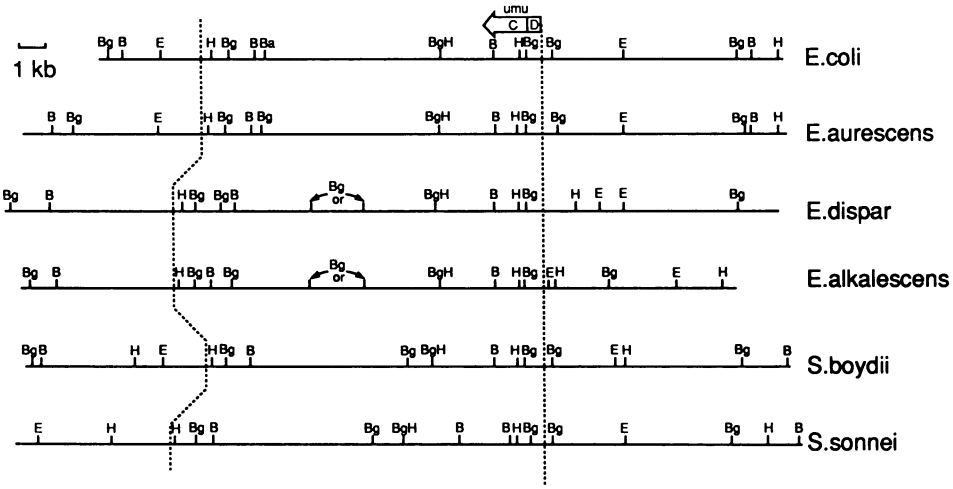

FIG. 4. The *umuDC* regions of *E. coli*, *E. aurescens*, *E. alkalescens*, *E. dispar*, *S. boydii*, and *S. sonnei* are conserved. Partial restriction site maps of the *umuDC* regions of these species were constructed from Southern hybridizations of genomic DNA digested with *Eco*RI, *Bam*HI, *Hin*dIII, and *Bgl*II in pairwise combinations. The dotted lines denote a tract of restriction sites common to all species. Stocks used were *E. coli* AB1157, *E. dispar* NCTC 4168, and *E. alkalescens* NCTC 1601.

response induces synthesis of the 15-kDa UmuD protein and then triggers autocatalytic conversion of UmuD to the smaller 12-kDa UmuD′ form that is active in mutagenesis. These events can now be visualized in *E. coli* cell extracts by using a newly developed chemiluminescent Western blotting technique (Tropix). Using polyclonal antibodies raised against highly purified UmuD and UmuD′ proteins, we have been able to detect hitherto undetectable levels of chromosomally encoded UmuD protein (51). *E. coli* induced for SOS with mitomycin C shows induction of the full-size UmuD protein and the shorter UmuD′ cleavage fragment (Fig. 7). The presence of similar amounts of other cross-hybridizing material in the induced and uninduced samples serves as an internal control to show that more protein was not applied in the induced extracts.

The occurrence of SOS induction of UmuD- and UmuD′-like proteins in other enterobacteria was also demonstrated (Fig. 7). In *E. dispar*, *E. aurescens*, *E. alkalescens*, *E. vulneris*, *S. sonnei*, and *S. boydii*, mitomycin C treatment induced UmuD- and UmuD′-like proteins with sizes similar to those detected in *E. coli*. Most UmuD and UmuD′ were detected in *E. dispar*, where it was even possible to detect a low level of UmuD in untreated cells. The cleavage reaction producing UmuD′ appeared to be most efficient in *S. typhimurium*, where the induced protein was mainly in the

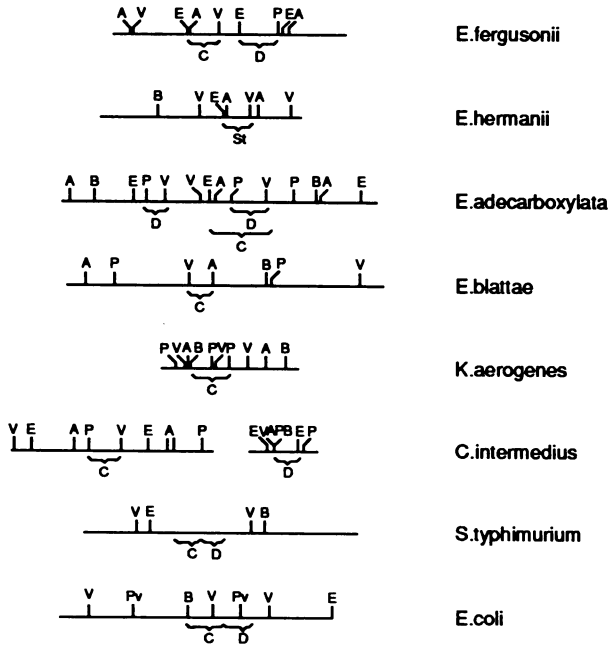

FIG. 5. *umu*-like regions of enterobacteria. Partial restriction maps of the species indicated were constructed from Southern hybridizations of genomic DNA digested with *Pst*I, *Eco*RV, *Ava*II, *Bam*HI, and *Eco*RI in pairwise combinations. DNA probes were *umuD* and *umuC* fragments of *E. coli* and a *umuDC* region of *S. typhimurium* DNA. Minimum-size fragments homologous to *umuD* or *umuC* are bracketed. *umuDC* DNA of *E. coli* and *S. typhimurium* restriction maps are based upon results from other sequencing studies (45, 48).

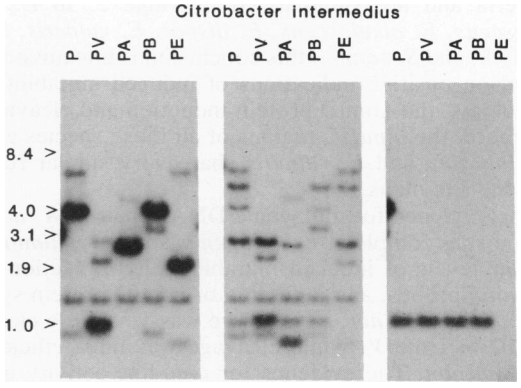

FIG. 6. Complexity of *umu* homology in *C. intermedius*. Sections of multiple digests of *C. intermedius* DNA were probed with *E. coli umuD* or *umuC* or with *S. typhimurium umuDC* DNA. P, *Pst*I; V, *Eco*RV; A, *Ava*II; B, *Bam*HI; E, *Eco*RI.

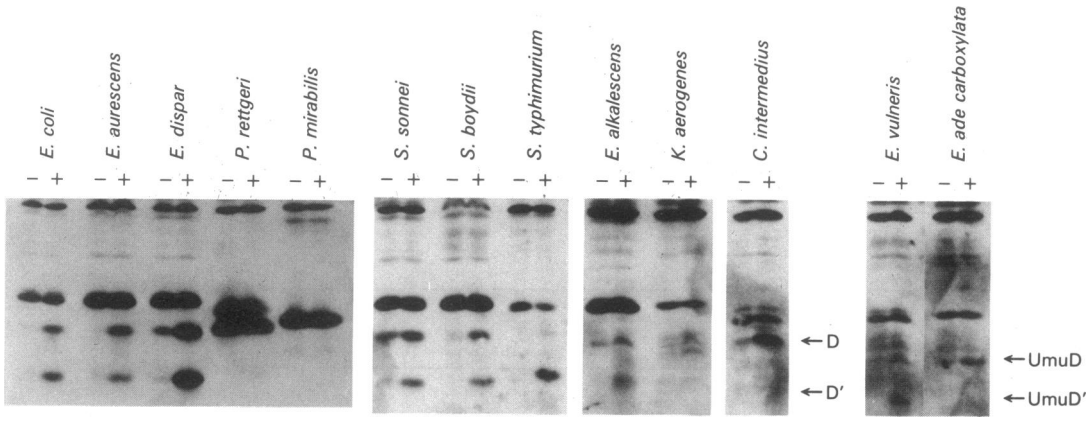

FIG. 7. Induction and cleavage of UmuD in enterobacteria. The bacterial species indicated were grown in the presence (+) or the absence (−) of 1 μg of mitomycin C per ml. Cell lysates were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western hybridization with polyclonal UmuD and UmuD' antibodies and a chemiluminescence assay. The positions of the full-size UmuD protein and its carboxy-terminal fragment produced by autocleavage are indicated.

cleaved form. Conversely, cleavage to the UmuD' form was poor or undetectable in *K. aerogenes*, *C. intermedius*, and *E. adecarboxylata*, even though synthesis of the full-size UmuD protein was induced. In the two *Proteus* species no induction of UmuD protein or UmuD' fragment was detected, although there were increased amounts of cross-hybridizing material of slightly higher molecular weight in both noninduced and induced cells of both species.

No UmuD- or UmuD'-like proteins could be detected reproducibly in mitomycin C-treated *E. blattae*, *E. fergusonii*, or *E. hermanii* (data not shown). UmuD and UmuD' antibodies were also unable to recognize analogous MucA and SamA proteins, which are 41 and 50% identical, respectively (data not shown). *S. typhimurium* UmuD protein, which is recognized by the *E. coli* UmuD and UmuD' antibodies, is 71% identical to its *E. coli* counterpart.

Thus, SOS induction of UmuD-like proteins is widespread in enteric bacteria. The cleavage of UmuD to UmuD' also occurs, but with different efficiencies.

DISCUSSION

Collectively, these results provide evidence for a widespread distribution of *umu*-like genes and activities in enteric bacteria and are summarized in Table 2. In *E. coli*, *E. aurescens*, *E. alkalescens*, *E. dispar*, *E. vulneris*, *S. typhimurium*, and *S. sonnei*, this conclusion is unequivocal and is based on positive indications of induced mutability, DNA homology, and UmuD protein induction and cleavage. Furthermore, the *umuDC* regions of all these species except *S. typhimurium* and *E. vulneris* share very similar restriction enzyme site maps.

The evidence for mutagenic DNA repair in *K. aerogenes* is almost as complete. *K. aerogenes* and *S. typhimurium* had similar levels of induced mutability, hybridization with *E. coli umu* probes, and induction of UmuD protein synthesis. However, in *K. aerogenes* there was very little cleavage of UmuD to UmuD', while cleavage was most efficient in *S. typhimurium*. The evidence for *umu*-like activity in *E. fergusonii* and *E. hermanii* rests on the presence of an inducible mutagenesis response and DNA homology. The inability to detect UmuD-like proteins may therefore reflect differences in UmuD proteins that make them unrecognizable by *E. coli* UmuD and UmuD' antibodies.

A second class of species could also be distinguished in which mutagenesis was very poor even though there were strong indications of the presence of *umu*-like genes and proteins. The nonmutability of *S. boydii* is particularly striking since *S. boydii* has the same restriction map of the *umuDC* region as *E. coli* and has UmuD-like protein and cleavage. *E. adecarboxylata* and *C. intermedius* also have *umu*-like DNA and induce UmuD-like proteins, but in these species cleavage to UmuD' was poor or undetectable and may be the basis of poor mutability. The discordance between the presence of *umu*-like DNA and mutagenesis occurred even between isolates of the same species. Multiple isolates of *E. coli*, *E. alkalescens*, and *E. dispar* displayed up to 200-fold differences in induced mutability, and yet all of them appear to have *umuDC* DNA (38) and are SOS inducible as judged by induction of RecA protein synthesis (data not shown). It is possible that in some cases sensitization and loss of mutations occurs through events like lysogenic induction. However, in *E. coli* such induction does not exclude mutagenesis in the fraction of cells surviv-

TABLE 2. Evidence for mutagenic repair in enterobacteria

Species	Induced mutagenesis	*umu* DNA homology	UmuD protein:	
			Induction	Cleavage
E. coli	+++[a]	+++	+++	+++
E. dispar	+++[a]	+++	++++	++++
E. alkalescens	+++[a]	+++	++	+++
E. aurescens	+++	+++	+++	+++
E. adecarboxylata	−[b]	+	++	−
E. blattae	−	+	−	−
E. fergusonii	++	+	−	−
E. vulneris	++	+	++	+++
E. hermanii	++	+	−	−
S. sonnei	+	+++	+++	+++
S. boydii	−	+++	+++	+++
K. aerogenes	+	+	++	+
S. typhimurium	+	+	+++	++++
P. mirabilis	−	−	?	−
P. rettgeri	−	−	?	−
C. intermedius	−	+	+++	−

[a] Highest levels, range of induced mutability in different isolates.
[b] −, not detectable but may be present.

ing DNA-damaging treatment and lysogenic induction (unpublished observation).

The evidence for mutagenic repair in *E. blattae*, *P. mirabilis*, and *P. rettgeri* was the least revealing. No induced mutations could be detected in these species. In *E. blattae* there was a low level of DNA homology, although this was considered to be *umu* specific according to the criterion of homology with nonoverlapping probes. However, no DNA homology could be detected in either *Proteus* species. No induction of UmuD-like protein was seen in these three species, and so it remains an open question as to whether UmuD proteins are absent or are simply not recognized by the *E. coli* UmuD and UmuD′ antibodies. In the two *Proteus* species there was an additional band of protein present in untreated and induced cells which had no counterpart in other species, and so it is not known whether in these organisms there is constitutive synthesis of UmuD-like protein.

In species such as *E. adecarboxylata*, *E. vulneris*, and *C. intermedius*, the patterns of *umu*-specific hybridization were complex. That of *E. adecarboxylata* generated a restriction map with two linked regions of different *umuD* homologies and at least one intervening tract of homology with *umuC*. In *E. vulneris* a single contiguous restriction map could not be generated, and it is possible that two separate regions of *umu* homology exist in this organism. The complexity of *umu* homology was most marked in *C. intermedius*, and further analyses are needed to resolve fully the multiple patterns of hybridization into DNA restriction maps. The idea that an organism can have more than one mutagenic DNA repair operon and yet remain only modestly mutable is tenable since the discovery that *S. typhimurium* LT2 has at least two mutagenic DNA repair operons (30). Furthermore, the environs of the *umu* genes in *E. coli*, *E. alkalescens*, *E. dispar*, and *S. typhimurium* have been found to be polymorphic (38) and may on occasion produce rearrangements in the *umu* genes.

In describing the *umu*-like genes and proteins of enteric species, it has been tacitly assumed that they are chromosomal in origin. Nevertheless, the presence of analogous genes on conjugative plasmids is well documented (for a review, see reference 47). Therefore, it is possible that some of the *umu*-like genes and proteins analyzed here are derived from plasmids. However, of the five *umu*-like operons isolated so far, DNA hybridization has been found only between the two genomic *umu* operons of *E. coli* and *S. typhimurium*. No hybridization has been reported between the plasmid *impCAB*, *mucAB*, or *samAB* loci. Similarly, the antibodies to *E. coli* UmuD and UmuD′ used here do not recognize the plasmid MucA or SamA proteins, which are 41 and 50% identical, respectively. The only cross-reacting protein with known identity is *S. typhimurium* UmuD, which is 71% identical to its *E. coli* counterpart. Amino acid sequence comparisons of the two chromosomal Umu operons place them much closer together than the three more diverged plasmid operons. Thus, it is more likely that the *umu*-specific DNA homology and antigenicity is derived from chromosomal rather than plasmid mutagenic DNA repair genes.

The general picture emerging from this survey is that there are indeed *umu*-like genes and proteins in a wide variety of organisms matching the widespread conservation of the SOS response. Further evidence for the wider dispersion of *umu*-like genes has come with the identification of this mutagenic DNA repair operon in *Streptomyces coelicolor*, although it is not clear in this example whether a plasmid or chromosomal sequence has been identified (27).

This view contrasts with an earlier conclusion (35) that *umu*-like genes appeared to be undetectable in many species. Evidently the dot-blot hybridization employed in this earlier work was by no means as sensitive as the Southern hybridization approach used here with its *E. coli* and *S. typhimurium* probes. These contrasting results emphasize the need for a multifunctional approach for gene surveys of this type.

In some cases, the presence of *umu*-like DNA was associated with strong mutagenic responses to DNA damage, as in *E. coli*. However, in many cases there were positive indications of *umu*-like genes in organisms with widely differing mutabilities. Obviously, the induced mutability is not just an absolute reflection of *umu* activity in isolation. Rather, it is a composite product of Umu protein action coupled with the susceptibility of the target sequences in each organism to lesion formation, the activity of competing repair systems, and the multifarious interactions of UmuD, UmuD′, and UmuC, both with each other and with other cellular components such as RecA and possibly GroEL (3, 6, 10, 53). It is therefore likely that quantitative differences between different species might be produced if an alternative mutational assay system other than induction to rifampin resistance was used. Thus, the important feature of the mutational results presented here is that a range of mutagenic responses was seen rather than the absolute level of induced mutagenesis to rifampin resistance. Given these provisos, there remain several indications that the level of mutagenesis can be related in part to the potency of Umu-like activity. For instance, *S. typhimurium* has *umu*-like genes, it induces UmuD protein, and it cleaves UmuD protein most effectively of all the species examined (52), and yet it is less mutable than *E. coli* in several different mutational assays. Furthermore, introduction of *E. coli recA* into *S. typhimurium* did not increase induced mutability, suggesting that poor mutagenesis was not based on a limitation of *S. typhimurium* RecA action (unpublished observation). Different mutagenic potencies are also seen when cloned examples of *umu*-like genes are introduced into *E. coli* (4, 30, 37) or when *mucAB* enhanced the low levels of mutagenesis of many of the species examined here (35). Thus, *umu* operons from different sources may have different mutagenic potencies inherent in their sequences.

To date, five *umuDC*-like operons have been characterized at the molecular level (8, 20, 30, 32, 40, 45, 48). All share certain structural similarities that suggest that they originated from a common ancestor. This report suggests that other *umu* operons exist in several genera of bacteria. The acquisition of a *umu* operon therefore seems to have been advantageous in evolutionary terms. Is this advantage the result of the enhanced variability that the *umu* operon provides? If this were the simple answer, it is interesting that many of the presumedly chromosomally encoded *umu* operons seem to be defective in this function. The mutagenic activity of the plasmid-encoded *umu*-like operons also appears to be constrained. In TP110, the I-group plasmid that carries the *imp* operon, *impC*, appears to code for a protein that regulates the *impAB* mutagenesis genes (20). Similarly, a region of R46 deleted in the creation of pKM101 also constrains the mutagenic repair activity of the MucAB proteins (7, 25).

The notion that mutagenic DNA repair genes may be present but that they are curtailed in their mutagenic potency raises the possibility that these genes are retained, not only for mutagenesis but for some other cellular process which

remains to be elucidated. For instance, the recovery of DNA replication after UV irradiation of *E. coli* required *umu* activity in certain *recA* mutants (50); perhaps in other species this interaction of *recA*-like and *umu*-like proteins is the norm. Also, *umu* mutations in *E. coli* have recently been found to reduce SOS-induced alleviation of *Eco*K DNA restriction through some mechanism which can be genetically distinguished from induced mutagenesis (12). Whether one of these processes, or some other, is the selective drive for the retention of *umu*-like genes in species with a poor mutagenic response is an intriguing possibility.

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