JOURNAL OF BACTERIOLOGY, Sept. 1990, p. 4979–4987
0021-9193/90/094979-09$02.00/0

Vol. 172, No. 9

Structural Characterization of the *Salmonella typhimurium* LT2 *umu* Operon

SUSAN M. THOMAS,* HELEN M. CROWNE, SARA C. PIDSLEY, AND STEVEN G. SEDGWICK

Genetics Division, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom

Received 20 March 1990/Accepted 13 June 1990

The *umuDC* operon of *Escherichia coli* encodes functions required for mutagenesis induced by radiation and a wide variety of chemicals. The closely related organism *Salmonella typhimurium* is markedly less mutable than *E. coli*, but a *umu* homolog has recently been identified and cloned from the LT2 subline. In this study the nucleotide sequence and structure of the *S. typhimurium* LT2 *umu* operon have been determined and its gene products have been identified so that the molecular basis of *umu* activity might be understood more fully. *S. typhimurium* LT2 *umu* consists of a smaller 417-base-pair (bp) *umuD* gene ending 2 bp upstream of a larger 1,266-bp *umuC* gene. The only apparent structural difference between the two operons is the lack of gene overlap. An SOS box identical to that found in *E. coli* is present in the promoter region upstream of *umuD*. The calculated molecular masses of the *umuD* and *umuC* gene products were 15.3 and 47.8 kilodaltons, respectively, which agree with figures determined by transpositional disruption and maxicell analysis. The *S. typhimurium* and *E. coli umuD* sequences were 68% homologous and encoded products with 71% amino acid identity; the *umuC* sequences were 71% homologous and encoded products with 83% amino acid identity. Furthermore, the potential UmuD cleavage site and associated catalytic sites could be identified. Thus the very different mutagenic responses of *S. typhimurium* LT2 and *E. coli* cannot be accounted for by gross differences in operon structure or gene products. Rather, the ability of the cloned *S. typhimurium umuD* gene to give stronger complementation of *E. coli umuD77* mutants in the absence of a functional *umuC* gene suggests that *Salmonella* UmuC protein normally constrains UmuD protein activity.

Activity of the *umuDC* operon of *Escherichia coli* is essential for mutagenesis induced by a variety of physical and chemical agents (38, 52, 55). The two *umu* genes encode an uncharacterized activity which increases the ability of the cell to tolerate and repair damage at the expense of genetic fidelity (8, 15, 47). It is an interesting evolutionary feature of *umu* genes that analogous operons exist on many conjugative plasmids (10, 22, 23, 32, 39, 48, 50). Two such operons, *mucAB* and *impCAB*, have been cloned from plasmids pKM101 and TP110, respectively (10, 31).

Expression of *umuDC*, *mucAB*, and *impCAB* genes is under the control of the SOS response (1, 6–8, 41). Under normal cellular conditions, transcription of these genes is repressed by the presence of LexA protein bound to a consensus binding sequence in the operator region of each operon (52). When DNA damage occurs, LexA repressor undergoes autoproteolytic cleavage by interaction with a complex of RecA protein, single-stranded DNA, and ATP (17, 43), and transcription of SOS-inducible genes ensues. However, for full mutagenic repair activity, the UmuD protein of *E. coli* or the MucA protein must itself be cleaved by interaction with the same "activated" RecA protein complex (3, 9, 21, 25, 40). The autoproteolytic cleavage of LexA, UmuD, and MucA proteins, as well the related cleavage of λ and P22 repressors, occurs at single conserved sites and requires equally well conserved catalytically active residues elsewhere in the molecules (5, 17, 36, 43, 44). The role of the activated RecA protein complex in cleavage is as a cofactor rather than as the proteolytic entity itself (17).

Recently, a *umuDC*-like operon provisionally designated *umuST* was identified (45, 49) and cloned (49) from the LT2 subline of the closely related species *Salmonella typhimurium*. The newly characterized operon is of interest because the mutagenic response of *S. typhimurium* LT2 is weak compared with that of *E. coli*. Many previous studies show that UV induces a much lower level of mutagenesis in *S. typhimurium* LT2 than in *E. coli* and that Weigle reactivation in *S. typhimurium* LT2 is also poor (13, 18, 28, 29, 51). Both reduced mutagenesis and Weigle reactivation of *S. typhimurium* can be restored to near normal levels by plasmids carrying the *E. coli umuDC* operon or analogous plasmid operons (2, 49, 51). Furthermore, transduction of the *E. coli umuDC* region into the *S. typhimurium* genome increased mutability (42). All of these observations point to a possible defect within the *S. typhimurium umu* operon itself which leads to an inefficient mutagenesis mechanism.

Thus, to investigate the molecular basis of this interspecies difference in mutagenesis and to further understand induced mutagenesis in general, we have structurally characterized the *S. typhimurium umu* operon. This paper presents the nucleotide sequence and protein products of the operon and discusses possible reasons for its weaker phenotype with respect to its *E. coli* counterpart.

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacterial strains are described in Table 1.

Plasmids pSE117 and pLM207 carry the *E. coli umuDC* operon (8, 20). pLM205 encodes only *E. coli umuD* (20). Plasmid pMH1291, which carries the *S. typhimurium umuDC* operon, was made by subcloning a 2.2-kilobase-pair (kbp) *Hin*dIII-*Sal*I fragment from pMH2532 (49) into *Hin*dIII- and *Sal*I-cut pBR322. pMH1300 contains the 0.9-kbp *Sal*I-*Xho*I fragment of pMH1291 cloned into *Sal*I-cut pBR322 (see Fig. 1). All plasmids were maintained routinely in *E. coli* DH5 (12) at 42°C to prevent rearrangements and cold sensitivity (20).

* Corresponding author.

TABLE 1. Bacterial stocks

Strain	Genotype	Source or reference
DH5	F⁻ *endA1 hsdR17* (r_K^- m_K^+) *thi-1* λ^- *recA1 gyrA96*	12
AB1157	*umu*⁺ *thr-1 ara-14 leuB6 lacY1* Δ(*gpt-proA*)*62 tsx-33 supE44 galK2 hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1*	14
CSR603	As AB1157 *recA1 uvrA phr-1*	34
GW2100	As AB1157 *umuC122*::Tn*5*	8
TK614	As AB1157 *umuD77 uvrA ilv-325 arg*⁺	15
MH1433	F⁺ *deoC srl*::Tn*10 recA1*(pMH1291)	This work

Media and culture. Luria-Bertani agar and broth were used for routine bacterial culture (19). They were supplemented when necessary with 50 μg of ampicillin (Sigma Chemical Co., St. Louis, Mo.) per ml for plasmid maintenance. UV-induced reversion of *his-4* mutants to His⁺ was detected on semienriched plates as previously described (49).

Transposon mutagenesis. Tn*1000* mutagenesis of pMH1291 was done by the F-factor mating technique of Guyer (11). Male *E. coli* MH1433 containing F and pMH1291 were mated with GW2100 *umuC122*::Tn*5*. Selective plates contained 50 μg of ampicillin per ml to select for transfer of pMH1291 and 100 μg of streptomycin per ml and no thymine to counterselect the donor. Insertions of Tn*1000* into the *umu* genes of pMH1291 were screened in patch plate assays of induced mutagenesis to histidine prototrophy (49). It was essential to use F⁺ and not F′ males in these constructions to avoid frequent plasmid rearrangements.

UV irradiation. Samples were irradiated with low-pressure mercury vapor lamps. Doses were determined with a UVX Radiometer (Ultraviolet Products, Inc., San Gabriel, Calif.).

UV-induced mutagenesis. Induced mutability was qualitatively screened by patching single colonies in 10-mm² squares on semienriched plates and irradiating them with 15 J of UV per m². UV-induced reversion to histidine prototrophy was detected as the appearance of small colonies within the spread patches after 3 days at 42°C. Quantitative assays of induced reversion to His⁺ prototrophy were as previously described (49).

Enzymes and biochemicals. Restriction enzymes and bacteriophage T4 DNA ligase were from Bethesda Research Laboratories, Inc., Gaithersburg, Md., unless otherwise specified. [γ-³⁵S]ATP and [³⁵S]methionine were purchased from Amersham International PLC.

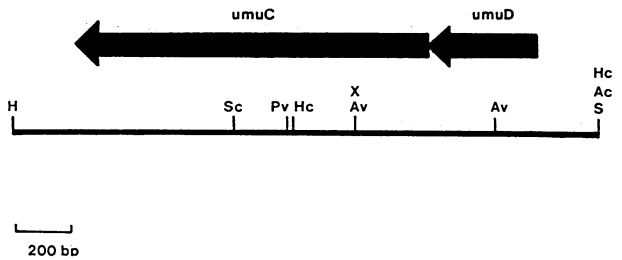

FIG. 1. Restriction map of the *S. typhimurium umuDC* operon contained with a 2.2-kilobase *Hin*dIII-*Sal*I fragment. Thick arrows show the deduced directions of transcription of the two genes. Abbreviations: H, *Hin*dIII; Sc, *Sac*I; Pv, *Pvu*I; Hc, *Hin*cII; X, *Xho*I; Av, *Ava*I; Ac, *Acc*I; S, *Sal*I.

Labeling of plasmid-encoded proteins in maxicells. The maxicell method of Sancar et al. (34) was used to radioactively label proteins produced by plasmids pSE117 and pMH1291 and a series of Tn*1000* insertion derivatives in strain CSR603. Samples were electrophoresed through a 12% sodium dodecyl sulfate-polyacrylamide gel and visualized by autoradiography.

General DNA methodology. All plasmid DNA was extracted from bacteria by the method of Chu and Berg (4) except that each preparation was twice spun to equilibrium in a cesium chloride gradient. All other techniques used were as described by Maniatis et al. (19).

DNA sequencing. The sequencing strategy was to use Tn*1000* insertions as mobile bidirectional priming sites so that only two primers were needed for divergent sequencing reactions on opposite strands from any point of random insertion. The sites and orientations of transposon insertions were mapped by individual pMH1291 *umu*::Tn*1000* stocks using the single asymmetric *Sst*I and *Bam*HI sites 73 and 387 bp, respectively, from the δ end of Tn*1000* (11). Insertions of Tn*1000* some 300 bp apart and of known transposon orientation were chosen. In this strategy, priming sites have to be chosen inside the 35-bp inverted terminal repeats of Tn*1000* (33) to be unique. δ primer, AGGGGAACTGAGAGCTCTA, is homologous to bases 86 to 68 of Tn*1000* and primes reactions out from the δ end of Tn*1000* into the flanking target DNA. Sequencing of the opposite DNA strand in the opposite direction was done with γ primer, CAGCTACAA CATACGAAAG, whose 3′ end is 70 bp from the γ terminus of Tn*1000*. Standard conditions of dideoxynucleotide sequencing (35) were used as specified by the U.S. Biochemical Corp. for Sequenase Sequencing Kits versions I and II. The sequencing strands first produce common Tn*1000* bands that end in the 35-bp inverted terminal repeat with its conspicuous final CCCC (33). These Tn*1000* sequences were removed by electrophoresis for 3 to 4 h at 1,500 V through a 48-cm-long gel, leaving up to 500 bp of readable target sequence extending from the transposon ends. The compilation of overall target sequence should allow for a 5-bp duplication of the sequence immediately flanking the ends of Tn*1000* (33).

Nucleotide and amino acid homologies between *E. coli umu*, *S. typhimurium umu*, and pKM101 *muc* operons were evaluated by both the Wilbur-Lipman (54) and the Needleman-Wunsch (24) algorithms.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper have been submitted to GenBank (accession no. M35010).

RESULTS

Subcloning of the *umu* operon. The initial clone of *S. typhimurium* DNA encoding mutagenic repair was a 3.9-kbp *Bam*HI-*Eco*RI fragment carried by pMH2532 (49). Prior to DNA sequencing, this fragment was reduced in size to a 2.2-kbp *Hin*dIII-*Sal*I fragment cloned into pBR322 and designated pMH1291. A more-detailed restriction map of the *umu* operon contained within the insert of pMH1291 is shown in Fig. 1. This map confirms our earlier observation that restriction sites within the *S. typhimurium umu* region differ from those of other homologous operons such as *umuDC* in *E. coli*, *impCAB*, and *mucAB*. The *Salmonella umu* region carried by pMH1291 continued to complement *E. coli umuD77* and *umuC122*::Tn*5* mutants, as judged by restoration of UV-induced mutagenesis (Fig. 2). However, levels of complementation were lower than those achieved

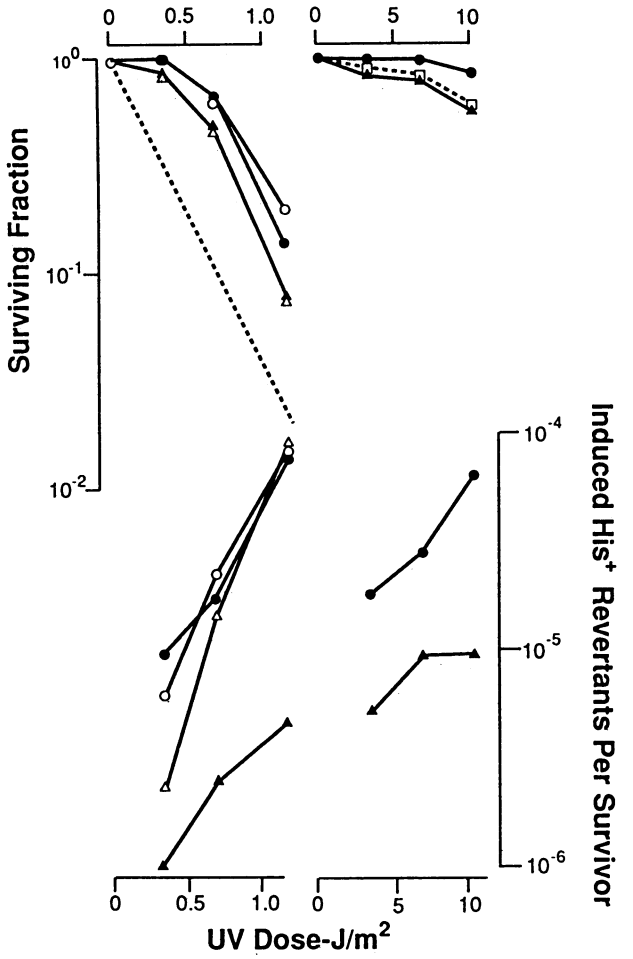

FIG. 2. Complementation of UV-induced mutagenesis and survival by cloned *S. typhimurium* or *E. coli umu* DNA in *E. coli uvrA umuD77* mutants (left) and *umuC122*::Tn*5* mutants (right). UV survival (top) and induced mutagenesis to histidine prototrophy (bottom) were tested in cells carrying plasmids pMH1291 *umuDC* (*S. typhimurium*) (▲), pMH1300 *umuD* (*S. typhimurium*) (△), pLM207 *umuDC* (*E. coli*) (●), or pLM205 *umuD* (*E. coli*) (○). Dotted lines show nontransformed hosts. Induced mutagenesis was undetectable in these and in the *umuC122*::Tn*5* mutant carrying *umuD* plasmids.

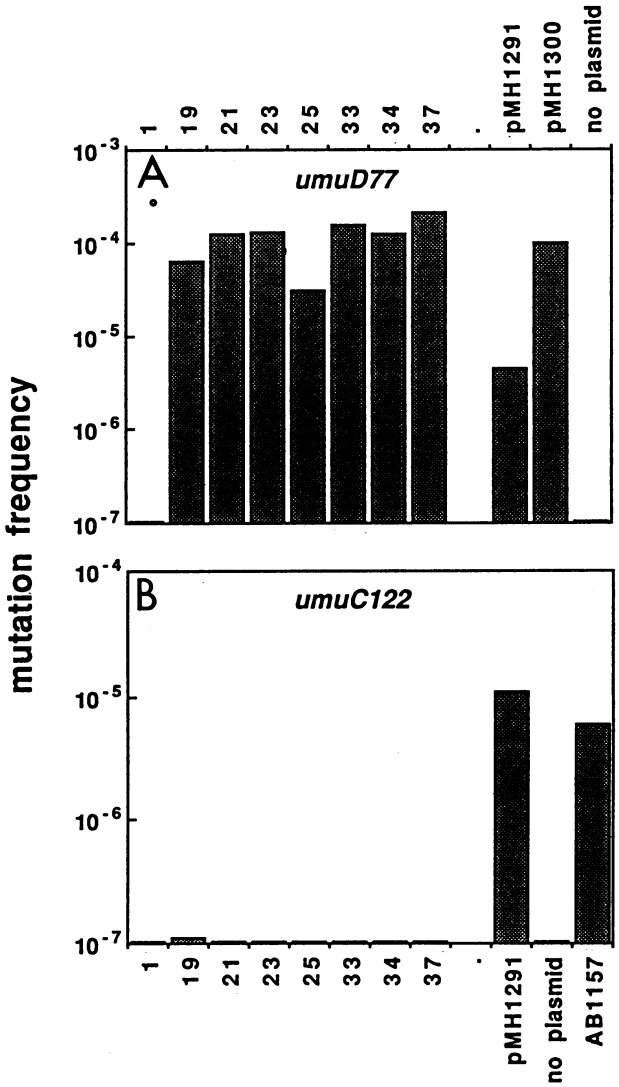

FIG. 3. Complementation of induced mutagenesis by Tn*1000* derivatives of pMH1291 in *E. coli umuD77 uvrA* (A) or *umuC122*::Tn*5* (B). Tn*1000* derivatives are numbered from 1 through 37. Control cells carried undisrupted pMH1291, a deletion derivative pMH1300, or no plasmid. *E. coli* AB1157 *umu*$^+$ is shown for comparison. His$^+$ mutagenic reversion was assayed after 0.7 J of UV per m^2 to *umuD77 uvrA* cells or 4.8 J of UV per m^2 to *umuC122*::Tn*5* *E. coli*.

by the comparable plasmid, pLM207, which carries the *E. coli umuDC* genes. As previously found (49), complementation of the *umuC* defect by pMH1291 was somewhat better than with the *umuD77* mutation (Fig. 2). Thus the relative mutabilities of these two species were qualitatively reflected by the complementing activities of their cloned genes.

Tn*1000* insertion mutagenesis of the *umu* operon. A pool of random Tn*1000* insertions within pMH1291 was generated by the F-factor mating technique of Guyer (11). Clones containing Tn*1000* inserted within the *S. typhimurium* mutagenic-DNA repair sequences were distinguished from others carrying Tn*1000* elsewhere in the vector by the absence of complementation of a *umuC122* mutation in *E. coli* GW2100. Following initial screening on patch plates, phenotypic disruption caused by Tn*1000* insertion was quantified in a subset of nonmutable GW2100(pMH1291::Tn*1000*) clones. For clarity, Fig. 3 presents induced mutation frequencies after a single dose, since essentially similar conclusions could be drawn from the full-dose-response assays actually carried out for each isolate. Survival levels were within the range of 54 to 100% for this experiment. Controls with nontransposed pMH1291 showed complementation of the *umuC122*::Tn*5* defect so that induced mutability was at levels similar to those for Umu$^+$ *E. coli* AB1157. As expected, the eight putative pMH1291 *umu*::Tn*1000* insertion plasmids did little to increase the frequency of induced mutagenesis in *umuC122*::Tn*5* *E. coli* GW2100 (Fig. 3b) and so were designated UmuC$^-$. pMH1291::Tn*1000* derivative 1 also failed to complement the *umuD77* mutation of *E. coli* TK614 and so was further designated UmuD$^-$C$^-$. All the other UmuC$^-$ derivatives, except derivative 1, increased mutagenesis from the undetectable levels of the nontransformed host (Fig. 3A) and were designated UmuD$^+$C$^-$. Mutagenesis was in fact increased to higher levels than with intact pMH1291. Similarly, higher levels of induced muta-

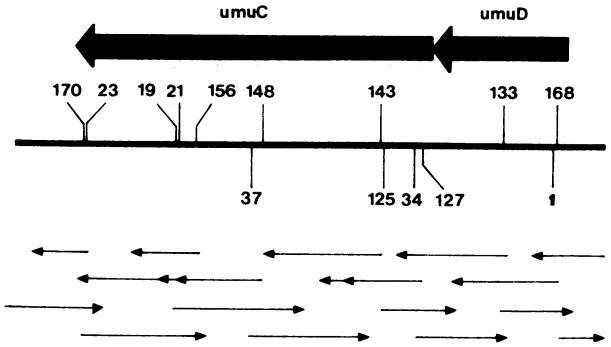

FIG. 4. Sites of Tn*1000* insertions within the *S. typhimurium umuDC* operon and their subsequent use in a double-stranded sequencing strategy (thin arrows) using primers to γ and δ ends of the transposon. Numbers above and below the line indicate opposite orientations of Tn*1000* within the operon. Thick arrows represent the directions of transcription of the *umuD* and *umuC* genes.

genesis were produced with pMH1300, which carries only the 0.9-kbp *Sal*I-*Xho*I segment of pMH1291 (Fig. 1) and which by similar genetic tests was designated UmuD$^+$C$^-$. This increase in complementing activity caused by deletion or insertion mutagenesis of the cloned *Salmonella umuC* sequences produced mutation frequencies similar to those achieved with *E. coli umuD* or *umuDC* plasmids (Fig. 2). Thus, UmuD$^+$ complementation by the cloned *Salmonella* DNA appeared to be more potent in the absence of accompanying UmuC$^+$ activity. Neither pMH1300 nor the *E. coli umuD* plasmid pLM205 complemented the *umuC122*::Tn*5* mutation (data not shown).

The orientation sites of Tn*1000* insertions in these and other transconjugants were mapped by reference to the *Bam*HI and *Sst*I sites near the δ terminus of the transposon and the *Hin*dIII and *Sal*I sites at the ends of the insert (Fig. 4).

Maxicell analysis of proteins. The proteins encoded by a subgroup of the Tn*1000* insertion mutants of pMH1291 together with the parent plasmid were examined in maxicells. Control *E. coli umuDC* plasmid pSE117 encoded two proteins of 16 and 46 kilodaltons (kDa) which from previous work are known to be the *umuD* and *umuC* gene products, respectively (8, 41) (Fig. 5). The *S. typhimurium umu* plasmid pMH1291 also encoded two proteins with similar electrophoretic mobilities and approximate molecular weights of 16 and 46 kDa. Tn*1000* derivatives 19, 43, 21, 34, 33, and 38, which were designated UmuD$^+$C$^-$ above, specified the 16-kDa UmuD-like protein but did not produce an equivalent 46-kDa UmuC-like gene product. Derivative 23, although phenotypically UmuD$^+$C$^-$, appeared to produce a full-size UmuC-like protein. However, the precise position of the Tn*1000* insertion of derivative 23 is so close to the 3′ end of the coding sequence (see below) that a truncated product would be produced that would be indistinguishable from a full-size product by electrophoresis. Derivative 1, which was phenotypically UmuD$^-$C$^-$, failed to produce either the 16- or the 46-kDa protein of the parent plasmid pMH1291. Thus, the UmuC$^-$ phenotype is associated with the loss of a 46-kDa plasmid-encoded protein, while UmuD$^-$C$^-$ incurs the loss of both 16- and 46-kDa gene products. The universal band at approximately 30 kDa represents the β-lactamase product of the *bla* gene, which was the selectable marker on all of the plasmids used for this experiment.

With these data, the restriction map of Tn*1000* insertion

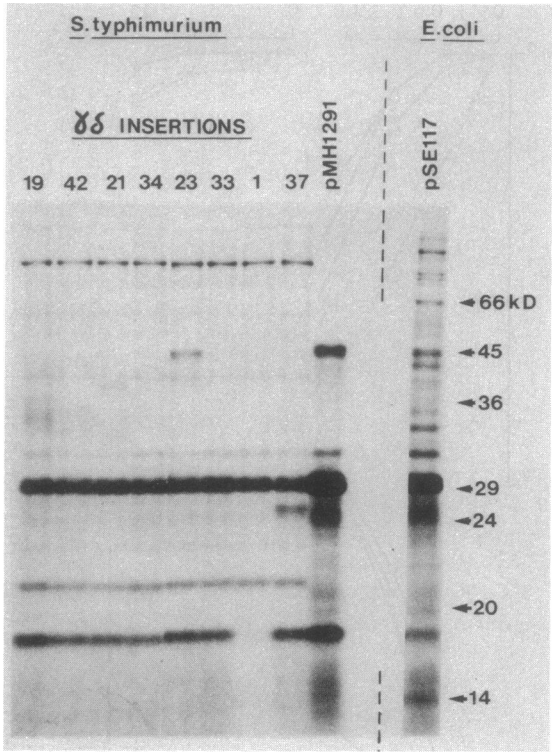

FIG. 5. [^{35}S]methionine-labeled proteins produced in maxicells from pSE117 (*umuD*$^+$*C*$^+$ of *E. coli*), pMH1291 (*umuD*$^+$*C*$^+$ of *S. typhimurium*), and several derivatives carrying Tn*1000* (γδ) insertions in pMH1291. Molecular size markers are indicated to the right. Samples were electrophoresed through a 12% sodium dodecyl sulfate-polyacrylamide gel and visualized by autoradiography.

sites, and the genetic complementation data, the organization of the *umuDC*-like operon can be envisaged. Transposon insertions are polar. Therefore derivative 1 has a polar insertion in a *umuD*-like analog so that it produced neither UmuD-like or UmuC-like proteins and did not complement *umuD* or *umuC* mutants. Derivatives which produced the 16-kDa protein but lacked the 46-kDa protein complemented *umuD* but not *umuC* mutants and so carry Tn*1000* insertions downstream from *umuD* in a *umuC*-like analog. The direction of transcription was therefore *umuD* and then *umuC*, commencing near the *Sal*I site end of the insert and terminating towards its *Hin*dIII end (Fig. 4). Since *S. typhimurium umu* clearly provided an activity identical to that of the equivalent *E. coli* genes and with similar-sized proteins, we shall henceforth refer to it as the *umuDC* operon.

DNA sequencing of the *umuDC* operon. In an alternative strategy to either primer leaping or subcloning strategies, a subpopulation of Tn*1000* insertions along the *Hin*dIII-*Sal*I insert of pMH1291 were used as multiple priming sites for DNA sequencing. The extent of sequences generated from each mapped transposon insertion is shown in Fig. 4.

The resultant nucleotide sequence of the 2.2-kilobase *Hin*dIII-*Sal*I fragment of pMH1291 which carries the *S. typhimurium umuDC* operon is shown in Fig. 6. It contains two open reading frames of 417 and 1,266 nucleotides which potentially encode proteins of 139 and 422 amino acids with calculated molecular weights of 15.3 and 47.8 kDa, respectively. Upstream of the smaller open reading frame is an SOS box consensus sequence for LexA protein repressor binding and a potential ribosome-binding site (16). The last

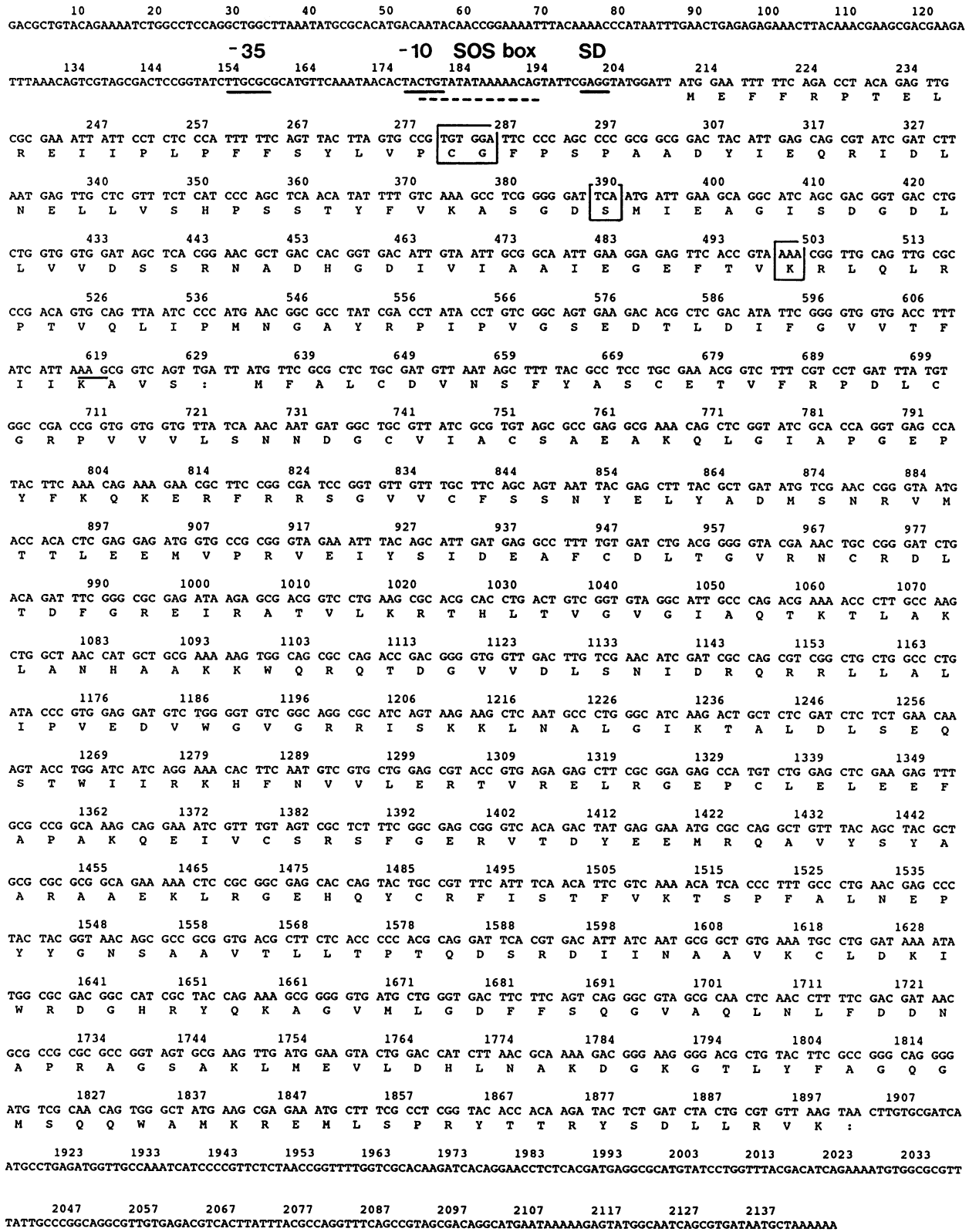

FIG. 6. Nucleotide sequence of the 2.2-kilobase *Hin*dIII-*Sal*I fragment of pMH1291 containing the *S. typhimurium umuDC* operon. The *umuD* coding sequence begins at the ATG initiation codon at bp 211 and terminates with TGA at bp 630. The *umuC* coding sequence begins at the ATG codon at bp 633 and terminates with TAA at bp 1898. The possible promoter elements, including the −35 and −10 regions and the Shine-Dalgarno sequence, are underlined. The SOS box consensus sequence for LexA repressor binding is between bp 178 and 194 and is marked with a broken line. The potential Cys-Gly cleavage site and associated catalytic sites are boxed.

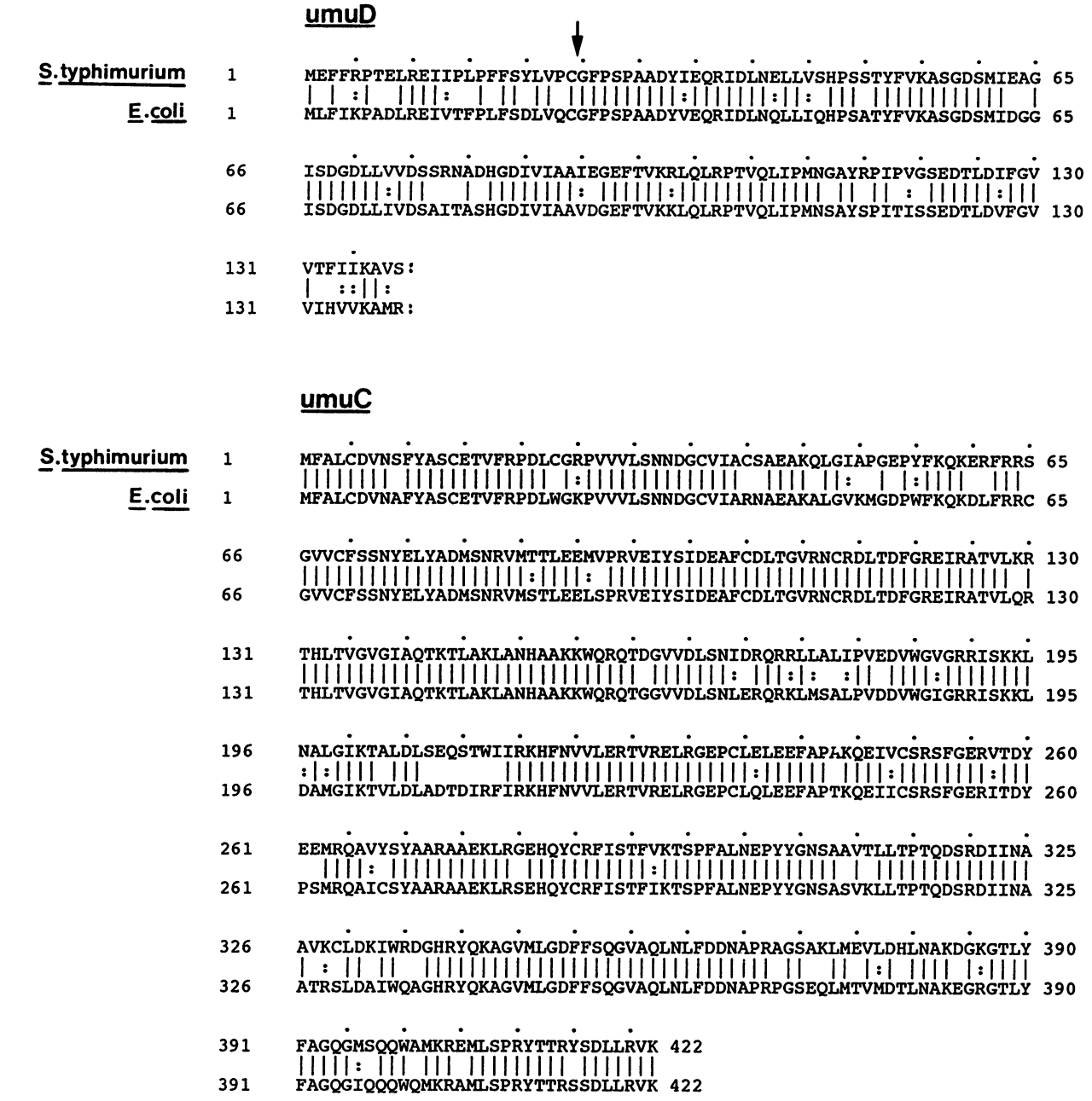

FIG. 7. Amino acid homology between the *E. coli* and *S. typhimurium umuDC* operons (24). Identical matches are shown by a solid line, and similarities are shown by a pair of dots; the arrow shows the UmuD cleavage site.

base of the termination codon of the small open reading frame for the *umuD* gene is 2 bases upstream of the initiation codon of the second larger open reading frame encoding UmuC. As with *E. coli*, the *S. typhimurium* UmuD sequence contains a Cys-Gly bond between amino acids 24 and 25. At this site the *E. coli* UmuD protein is autocleaved by RecA protein and activated for its role in mutagenesis (3, 25, 40). In addition, potential catalytic sites identified in the Slilaty-Little model (43) at Ser-60 and Lys-97 are conserved with respect to *E. coli*. Figure 7 compares matches of exact and similar amino acids in the *E. coli* and *S. typhimurium umuD* and *umuC* gene products (54). When the two sets of genes were aligned as in previous studies (30) to show the percentage of exact matches only (24), then the two *umuD* genes had 68% nucleotide homology and 71% amino acid identity (Fig. 8). It is interesting to note that there was approximately 20% more sequence divergence N terminal to the Cys-Gly cleavage sites of the two UmuD proteins in the part of the polypeptide which is presumably discarded prior to mutagenesis (3, 25, 40) (Fig. 7). The two *umuC* genes shared 71% nucleotide homology and 83% amino acid identity, with homologies distributed fairly evenly throughout the two genes (Fig. 7). The *S. typhimurium* operon also shared lower levels of homology with the *muc* genes, whose proteins play a similar role in plasmid-mediated mutagenesis (Fig. 8).

Using Tn*1000* as a mobile primer site for sequencing gave exact insertion sites for the transposon derivatives of pMH1291 used in the genetic and maxicell analyses de-

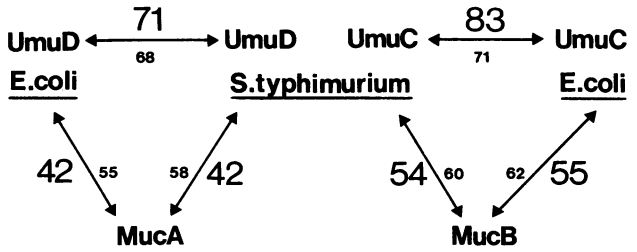

FIG. 8. Extent of exact DNA and protein matches between *mucA* and *mucB* and *S. typhimurium* and *E. coli umuD* and *umuC* genes. Small numbers, Percent DNA homology; large numbers, percent protein identity.

scribed above. Derivative 1 had an insertion 50 bp upstream of the initiation codon of the *umuD* structural gene (at bp 160; Fig. 6). Derivatives 19, 42, 21, 34, 33, 37, and 23 all had insertions in the *umuC* coding sequences, with that in derivative 23 being only 19 bp from the 3′ end. Thus derivative 23 would produce a truncated product indistinguishable from that of wild type by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the maxicell assay.

DISCUSSION

We have shown that mutagenic DNA repair in *S. typhimurium* is encoded by two genes organized in an operon with close similarities to the *E. coli umuDC* locus, the *mucAB* genes of plasmid pKM101, and the *impCAB* genes of plasmid TP110 (10, 16, 30). They all have a similar structural organization. With the exception of *impCAB*, they are dicistronic, with highly conserved SOS regulatory sequences and promoter regions preceding the smaller, UmuD-like open reading frames, which are followed by longer, UmuC-like coding sequences. Furthermore, all four *umuD*-like and *umuC*-like genes and their protein products are of a similar size. A minor difference in operon organization is that translation of *umuC* of *S. typhimurium* is displaced by +2 bp after the *umuD* stop codon into a different reading frame and so differs from the 1-bp overlap of the *E. coli umuD* stop codon and the start of *umuC* and the even larger 13-bp overlap of the *mucA* and *mucB* genes. Conventionally, translational coupling has been invoked in *umuDC* and *mucAB* (16, 30) and in polycistronic messages in general as a device for modulating the relative amounts of contiguous gene products (26, 27, 37). However, translational coupling remains to be directly demonstrated in these inducible mutagenesis genes, and so the significance, if any, of differing degrees of overlap to such coupling is unclear. The minor differences observed may simply result from compromises between the slightly different coding properties of the *umuD-umuC* junctions in the two species, the different relative stabilities of UmuD and UmuC proteins, and the structural constraints needed for access to the *umuC* Shine-Dalgarno sites whether by coupling or not.

Consistent with the physical similarities of nucleotide sequences, regulatory sequences, structural organization, and protein products, these operons have similar cellular activities. As with cloned *E. coli umuDC* (20, 41), *S. typhimurium umuDC* multicopy plasmids can restore induced mutagenesis to *E. coli umuD* and *umuC* mutants (49) although not as effectively as cloned *umuDC* genes from *E. coli*. It is therefore concluded that the role of the *S. typhimurium umuDC* operon in mutagenesis is identical to that of *E. coli umuDC*, although the phenotype in *S. typhimurium* is significantly weaker whether plasmid encoded or in situ in the chromosome, as judged by numerous reports of poor induced mutability of *S. typhimurium* (13, 18, 28, 29, 52).

Given these similarities with the active *E. coli umuDC* operon, the question of why *S. typhimurium* is less mutable than *E. coli* remains. Our previous study indicated that cloned *S. typhimurium umu* had a weaker ability to complement mutations in *E. coli umuD* than in *umuC* (49). In addition, hybridization of *S. typhimurium umu* to an *E. coli umuD* probe was very much weaker than hybridization to a *umuC* probe (45, 49). From these results it was hypothesized that the *S. typhimurium umuD* sequence might have diverged significantly more than that of *umuC* and would have a less-active product (49). Indeed, the stimulation of induced mutagenesis of *S. typhimurium* by cloned *E. coli umuD* alone has been used to argue that UmuD activity is either absent (13) or at least limiting (46).

In the work presented here it is notable that complementation of *E. coli umuD77* mutants was more effective with plasmids lacking *umuC* both by in vitro deletion and by Tn*1000* insertion than with plasmids with an entire *S. typhimurium umuDC* operon. Thus the *S. typhimurium umuD* gene does not appear to be particularly inactive, at least in the confines of *E. coli*. Rather, its activity appears to be curtailed by accompanying *S. typhimurium umuC* genes. The activity of Umu proteins in vivo appears to need RecA protein for some third role in addition to derepressing *umuDC* and activating UmuD protein (25). Indeed, more recent in vitro evidence points to physical associations of UmuD, UmuC, and RecA proteins (9, 56), so it is plausible that a potentially functional UmuD protein might be constrained through interaction with other proteins in multiprotein complexes. An underlying conclusion of the UmuD cross-complementation found in our study is that *E. coli* and *S. typhimurium umu* genes are interchangeable, unlike their *mucAB* counterparts (13, 30, 53) (a more detailed account of the interchangeability of mutagenic DNA repair genes will be presented elsewhere).

The potential activity of UmuD protein is also indicated by examination of the amino acid sequence translated from the *S. typhimurium umuD* DNA sequence. The amino acid sequence contains a Cys-Gly cleavage site for possible RecA-mediated autocleavage, which, in *E. coli*, produces a carboxy-terminal fragment of UmuD protein essential for mutagenic DNA repair (3, 25, 40). Furthermore the *S. typhimurium* UmuD protein contains the putative catalytic sites for autoproteolysis consistent with the serine protease model of Slilaty and Little (43) and at the same relative positions as in *E. coli* UmuD protein. Despite these similarities, it is still possible that the activation of the UmuD protein by *S. typhimurium* RecA is lower than the comparable reaction in *E. coli*. This could be a reflection of sequence divergence at other unrecognized parts of the *umuD* gene or minor differences in RecA protein in the two organisms and remains to be tested directly. However, the stimulation of *E. coli* mutagenesis by cloned *Salmonella umuD* suggests that activation can happen outside of the parent organism.

The initial idea that the *E. coli* and *S. typhimurium umuD* sequences had diverged more than the equivalent *umuC* genes clearly conflicts with the comparison of nucleotide sequences presented here, which shows no large difference in the extent of homology between the two *umuD* genes compared with that between the two *umuC* genes. However, the original weak hybridization of *umuD* might now be explained by its use of a *umuD* probe containing less-homologous flanking sequences.

It is possible that differences in the expression of *umu* genes from the two species cause their different mutagenic potencies. However, this seems unlikely because the two have identical SOS boxes preceding their *umuD* genes and because *S. typhimurium umuD* alone cannot complement as effectively as its *E. coli* homolog.

In summary, this study shows that an organism with a weak mutagenic DNA repair response still carries highly conserved sequences for genes involved in this process. The apparent contradictions in previous studies on the *umu* status of *S. typhimurium* (13) can now be explained by the facts that, unlike other DNA repair operons of this type, the UmuD and UmuC proteins of *S. typhimurium* and *E. coli* can cross-complement and *S. typhimurium* mutagenesis can be boosted by additional UmuD activity. In the light of this information, it will be interesting to determine the limiting factors in *S. typhimurium* inducible mutagenesis. More generally it is clear that phenotype is not an exact indicator of the presence or absence of *umuDC* genes and that parallel molecular assays are needed. This combined approach is now being applied in the search for *umu*-like genes from a variety of organisms.

In the course of this work we learned that Smith et al. were engaged in similar studies on the *S. typhimurium umuDC* operon (46). Both groups agree on the definitive sequence presented here and in the accompanying paper (46).

ACKNOWLEDGMENTS

We are indebted to R. Reed for allowing us to use his unpublished sequence of transposon Tn*1000*, which was instrumental in our sequencing strategy. We thank D. Sherratt for sending this sequence to us. We also thank M. Blanco, G. C. Walker, B. Bridges, and P. Strike for gifts of plasmids and bacterial stocks. We thank Eric Eisenstadt and colleagues for exchange and fruitful discussion of data before publication. We also thank K. Hiom, G. R. Banks, and B. Sedgwick for useful advice; A. Spanos for computer assistance; and D. Moss for typing the manuscript.

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