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Sequence Analysis and Mapping of the *Salmonella typhimurium* LT2 *umuDC* Operon

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In *Escherichia coli*, efficient mutagenesis by UV requires the *umuDC* operon. A deficiency in *umuDC* activity is believed to be responsible for the relatively weak UV mutability of *Salmonella typhimurium* LT2 compared with that of *E. coli*. To begin evaluating this hypothesis and the evolutionary relationships among *umuDC*-related sequences, we cloned and sequenced the *S. typhimurium umuDC* operon. *S. typhimurium umuDC* restored mutability to *umuD* and *umuC* mutants of *E. coli*. DNA sequence analysis of 2,497 base pairs (bp) identified two nonoverlapping open reading frames spanning 1,691 bp that were 67 and 72% identical at the nucleotide sequence level to the *umuD* and *umuC* sequences, respectively, from *E. coli*. The sequences encoded proteins whose deduced primary structures were 73 and 84% identical to the *E. coli umuD* and *umuC* gene products, respectively. The two bacterial *umuDC* sequences were more similar to each other than to *mucAB*, a plasmid-borne *umuDC* homolog. The *umuD* product retained the Cys-24–Gly-25, Ser-60, and Lys-97 amino acid residues believed to be critical for RecA-mediated proteolytic activation of UmuD. The presence of a LexA box 17 bp upstream from the UmuD initiation codon suggests that this operon is a member of an SOS regulon. Mu d-P22 inserts were used to locate the *S. typhimurium umuDC* operon to a region between 35.9 and 40 min on the *S. typhimurium* chromosome. In *E. coli*, *umuDC* is located at 26 min. The *umuDC* locus in *S. typhimurium* thus appears to be near one end of a chromosomal inversion that distinguishes gene order in the 25- to 35-min regions of the *E. coli* and *S. typhimurium* chromosomes. It is likely, therefore, that the *umuDC* operon was present in a common ancestor before *S. typhimurium* and *E. coli* diverged approximately 150 million years ago. These results provide new information for investigating the structure, function, and evolutionary origins of *umuDC* and for exploring the genetic basis for the mutability differences between *S. typhimurium* and *E. coli*.

In several bacterial species, including *Escherichia coli* and *Salmonella typhimurium*, DNA damage or other treatments that block DNA replication lead to the induction of the SOS response (reviewed in references 41 and 62; C. Mark Smith, Ph.D. thesis, Harvard University, Cambridge, Mass., 1989). In *E. coli*, this response results from the transcriptional induction of as many as 20 genes. Induction of the SOS regulon is initiated when activated RecA protein promotes the proteolytic cleavage of LexA, the SOS repressor protein (25). RecA activation leads within minutes to depletion of LexA and induction of the SOS loci (25, 46).

Induction of two SOS genes, *umuD* and *umuC*, is required for efficient UV mutagenesis in *E. coli* (20, 52). Mutations in *umuDC* reduce the UV mutability of *E. coli* by more than 100-fold, modestly increase the UV sensitivity of *E. coli*, and reduce UV-inducible reactivation of UV-irradiated λ bacteriophage (20). Posttranslational activation of UmuD via RecA-promoted proteolysis is required to yield a biologically active carboxy-terminal fragment, UmuD′ (6, 36, 51). UmuD′ was recently shown to bind stoichiometrically to UmuC (68). RecA, the heat shock genes *groEL* and *groES*, and DNA polymerase III holoenzyme are additional components of SOS mutagenesis (5, 11, 13, 36). The precise biochemical role for UmuD′ and UmuC in SOS mutagenesis is unknown. Given that the preponderance of SOS mutagenesis occurs at sites of DNA damage (12), it appears that the *umuDC* gene products enable stalled DNA polymerase to replicate past noninformational DNA lesions (4).

UV mutability is not a universal bacterial phenotype even among members of the family *Enterobacteriaceae* (14, 48, 62), and in only a few bacterial species have *umu*-like chromosomal sequences been detected either by functional criteria (2, 16) or by hybridization (55). Extrachromosomal loci encoding *umu*-like functions have, however, been identified among at least 10 different plasmid incompatibility groups from at least six different genera (32, 34, 42, 56, 60). Two such extrachromosomal loci, *mucAB* and *impAB*, have been characterized at the sequence level (40, 56) and found to be homologous to *E. coli umuDC*. *mucAB* and *impAB* are each 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.

S. typhimurium exhibits poor UV mutability and meager Weigle reactivation of UV-irradiated P22 phage (48, 53, 61). These phenotypes suggested that *S. typhimurium* may be either devoid of or deficient in *umuDC* (16, 48, 61). UV mutagenesis in *S. typhimurium* (39), though less pronounced than in *E. coli*, appears, nonetheless, to require inducible functions that are repressible by LexA (38). The enhancement of UV mutagenesis in *S. typhimurium* by a *umuD* clone from *E. coli* indicated the presence of an active *umuC* gene in *S. typhimurium* (16). Homologs to *umuDC* in *S. typhimurium* were recently detected (55) and cloned (58; Smith,

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TABLE 1. Bacterial strains, plasmids, and phage

Material	Relevant characteristics	Source or reference
Bacteria		
E. coli		
AB1157	*argE3*	G. C. Walker
GW3200	AB1157 *umuD44*	G. C. Walker
GW3198	AB1157 *umuC36*	G. C. Walker
S. typhimurium		
PM155	*leuD21*	P. Margolin
TT15242	*purB1879*::Mu dP; 25 min ccw[a]	N. Benson; 70
TT15241	*purB1879*::Mu dQ; 25 min cw	N. Benson; 70
TT15630	*tre-152*::Mu dP; 35.9 min cw	N. Benson; 70
TT15625	*tre-152*::Mu dQ; 35.9 min ccw	N. Benson; 70
TT15250	*zea-3666*::Mu dP; 40.5 min ccw	N. Benson; 70
TT15249	*zea-3666*::Mu dQ; 40.5 min cw	N. Benson; 70
Plasmids		
pSTumu	pBR322 (3) clone of *S. typhimurium umuDC*	This study
pSE117	pBR322 (3) clone of *E. coli umuDC*	29
Phage		
Lambda dash	Cloning vector	Stratagene

[a] The number is the map position of the Mu d-P22 insert. cw (clockwise) and ccw (counterclockwise) refer to the packaging direction of the Mu d-P22 insert with reference to the orientation of the *S. typhimurium* chromosome. Mu dP and Mu dQ are designations for the two orientations of the P22 genome inserted between the ends of Mu (70).

Ph.D. thesis). Here, we report the DNA sequence analysis, physical mapping, and biological characterization of the *S. typhimurium umuDC* operon.

Our results establish that the cloned *S. typhimurium umuDC* operon is biologically active and highly similar in sequence to its *E. coli* homolog. A major difference between the *umuDC* loci was map position. We found that *umuDC* in *S. typhimurium* was located 10 to 15 min clockwise of its position in *E. coli*, placing it very near one end of a region whose gene order in *S. typhimurium* is inverted with respect to the order in *E. coli*.

The accompanying paper by Thomas et al. (57) provides independent confirmation of the sequence analysis presented here and additional biochemical and physiological characterization of the *S. typhimurium umuDC* gene products.

MATERIALS AND METHODS

Strains and strain manipulations. Strains and their relevant characteristics are summarized in Table 1. Strain constructions were performed by using standard transformation (23) and P22HT transduction (9) procedures. All plasmid and phage constructions were confirmed via restriction digests.

DNA isolation and manipulations. Plasmid and phage DNAs were isolated and handled by standard techniques (28). All restriction enzymes were from New England Bio-Labs, Inc., Beverly, Mass., and were used as recommended by the supplier. Ligations were performed overnight at 16°C with T4 DNA ligase. DNA probes used in the library screening and characterization of clones were isolated and prepared as previously described (55).

Mapping procedures. *S. typhimurium* strains carrying mapped Mu d-P22 inserts (Table 1) were used to amplify specific regions of the bacterial chromosome. Growth, phage lysate preparation, and DNA preparations were performed as described by Youderian et al. (70).

Library construction. A pBR322 library was constructed by Robin S. Monroe and kindly provided by Nicholas Kredich. The library was constructed by ligating a partial *Sau*3A1 digest of *S. typhimurium* DNA into pBR322 cut with *Bam*HI. Details of the construction of this library have been presented elsewhere (33). The lambda library used to isolate the complete *S. typhimurium umuDC* operon was constructed with the lambda-dash cloning kit and procedure from Stratagene. Briefly, *S. typhimurium* genomic DNA was digested with *Eco*RI, size fractionated over a sucrose gradient to a range of 10 to 20 kilobases (kb), ligated into the *Eco*RI cloning site of the lambda-dash vector, and packaged into lambda phage capsids. The final library comprised over 400,000 recombinants and was screened without amplification. *Eco*RI was chosen to generate the fragments for cloning because of previous restriction mapping results, which indicated that both *umuD* and *umuC* were contained on an *Eco*RI fragment of approximately 12 kb.

Library screening and nucleic acid hybridizations. The pBR322 library was screened with a 2-kb probe spanning *E. coli umuDC* as previously described (55). The lambda-dash library was screened in the same fashion with a 2.4-kb *Eco*RI fragment derived from a partial clone of the *S. typhimurium umuDC* locus which was isolated from the pBR322 library as described above.

Hybridization of end-labeled (28) oligonucleotide probes to DNA from Mu d-P22 was performed on GeneScreen Plus (Dupont, NEN Research Products, Boston, Mass.) according to the instructions of the manufacturer. Labeled probes were purified on NACS · 52 Pre-Pak Columns (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) prior to use. Hybridization reactions were performed for 2 h at 58°C in 5 ml of buffer containing approximately 3×10^6 cpm (specific activities of labeled probes ranged between 1.3×10^6 and 5×10^6 cpm/pmol). Filters were washed twice for 15 min each at 58°C in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) containing 0.5% sodium dodecyl sulfate.

The following oligonucleotides were synthesized and used as specific hybridization probes for *icd* (59), *dadB* (65), *phoP* (31), and *umuC* (this work), respectively: 5′-GGCGGTATT CGCTCTCTG-3′, 5′-GGCAGTTGAAAGCGCTGC-3′, 5′-GGTGATCAACATCCCGCC-3′, and 5′-GCATGGTTAGC CAGCTTGG-3′. The *icd*, *dadB*, and *phoP* probes have a theoretical T_m of 58°C. The T_m for the *umuC*-specific probe was 60°C. The T_m for oligonucleotides was calculated as described by Wallace and Miyada (64).

Sequencing. Sequencing was performed with Sequenase as described by the supplier (United States Biochemical Corp., Cleveland, Ohio). DNA sequences were obtained by "walking" along the subcloned fragments with sequential synthetic oligonucleotide primers (18- to 20-mers). Selection of primers was based on the endmost, nonambiguous sequence generated from the preceding primer. Both strands were sequenced in this manner. Of the 2,497 base pairs (bp) sequenced, all but two noncoding regions—the 5′-terminal 41 bp and the 3′-terminal 304 bp—were determined on both strands. The antisense sequence from position 800 to 2364 (see Fig. 2) was determined from an M13 subclone of the 2.4-kb *Eco*RI fragment described above. The remaining antisense sequence was determined from this 4-kb *Eco*RI-*Bam*HI fragment cloned into pBR322. Sense sequence from position 895 to 2061 was determined from the pBR322 clone. Sense sequence from position −133 to 895 was determined

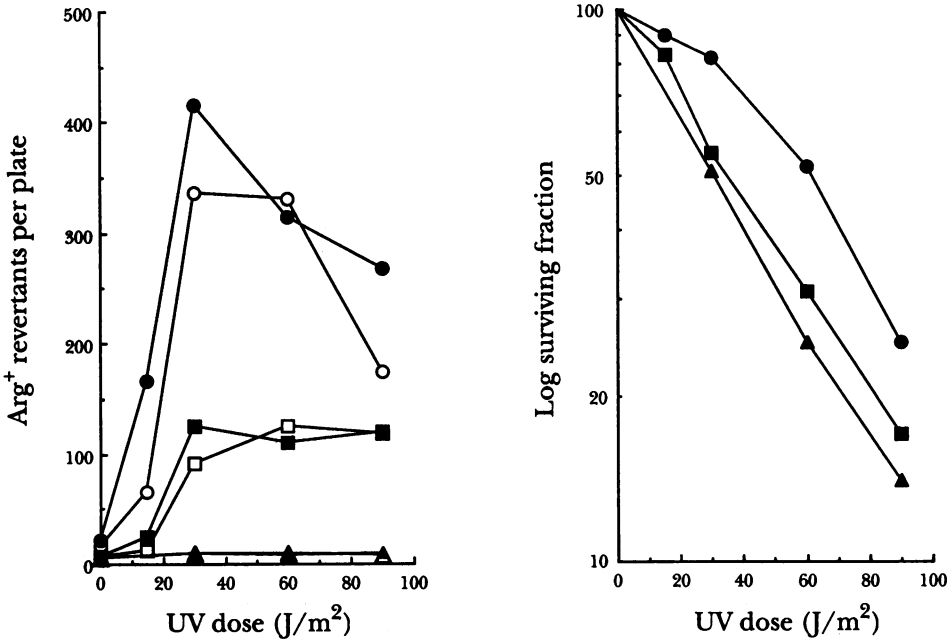


FIG. 1. Effects of *umuDC* clones from *S. typhimurium* or *E. coli* on mutability (left) and UV sensitivity (right) of *E. coli umuD* (open symbols) or *E. coli umuC* (closed symbols) mutants. Bacterial strains carrying pBR322 (△, ▲), a *umuDC* clone from *E. coli* (○, ●) or a *umuDC* clone from *S. typhimurium* (□, ■) were UV irradiated and plated for revertants or survivors as described in Materials and Methods. Survival was also determined for *E. coli umuD* (not shown) and was indistinguishable from the results obtained with *E. coli umuC*.

from DNA that had been asymmetrically amplified by the polymerase chain reaction (19). The same primers were used for the polymerase chain reaction and for sequencing the sense strand.

Culture media. Bacteria were grown in NB medium (nutrient broth no. 2; Oxoid Ltd., London) at 37°C unless otherwise noted. Minimal medium was M9 medium with 0.2% glucose, as described elsewhere (30), and 0.001% Casamino Acids (Difco Laboratories, Detroit, Mich.). Medium for plasmid-bearing strains contained either 100 or 25 μg of ampicillin per ml for NB and minimal media, respectively.

UV mutagenesis and killing. Bacteria containing plasmids were grown in minimal medium supplemented for all auxotrophies and containing 25 μg of ampicillin per ml. Overnight bacterial cultures were diluted 100-fold into fresh medium and grown to cell densities of approximately 2×10^8 cells per ml. From each culture, 4 ml was removed to large glass petri dishes and irradiated with constant agitation at the fluences noted. For each dose, samples were removed immediately, diluted into 0.85% NaCl, and plated for survivors on minimal plates. Arg^+ revertants were scored after a 3-day incubation at 37°C on minimal medium plates supplemented with 1 μg of arginine per ml.

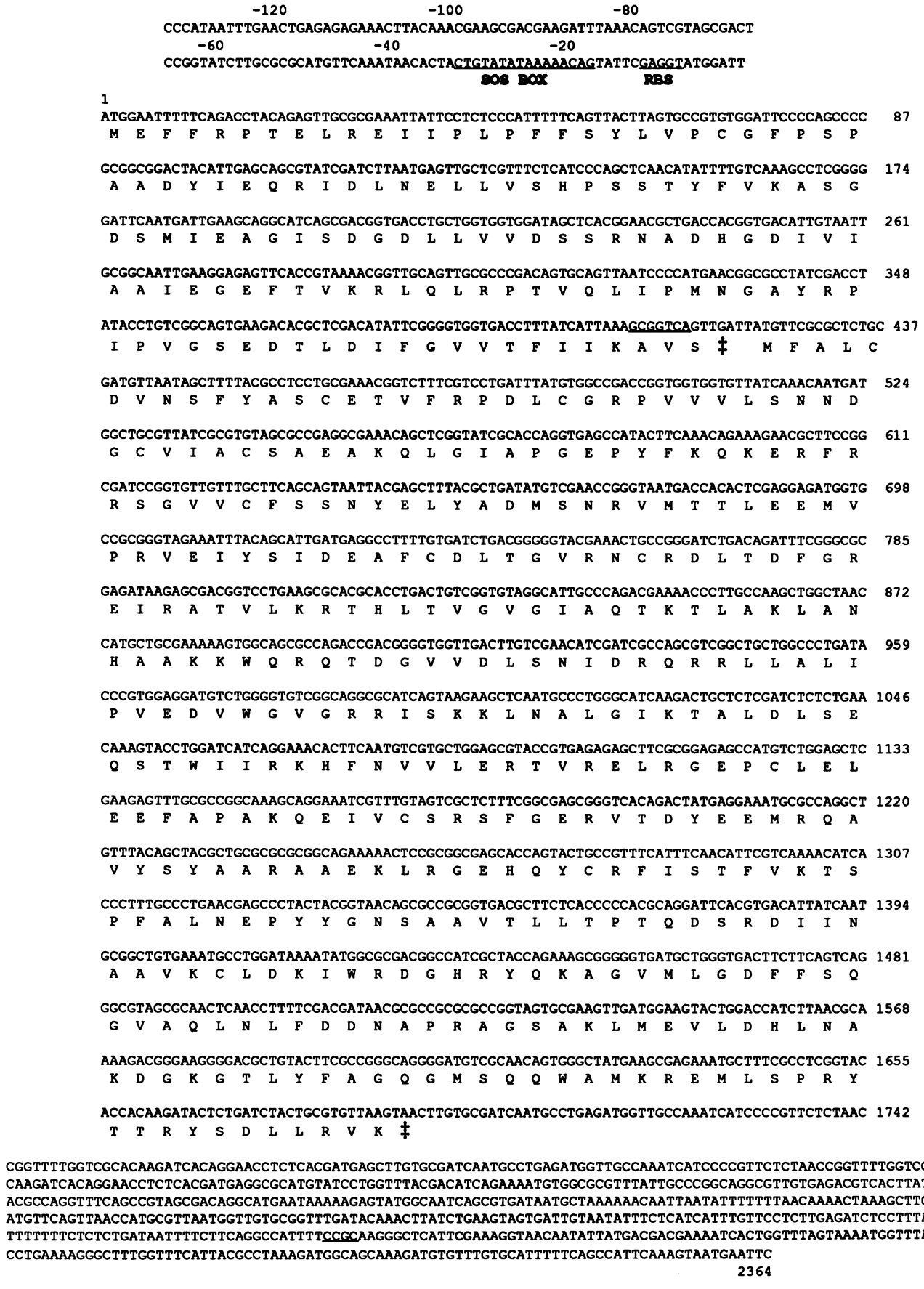
Nucleotide sequence accession number. The nucleotide sequence data reported in this paper have been submitted to GenBank (accession no. M35010).

RESULTS

Cloning the *S. typhimurium umuDC* operon. Approximately 34,000 recombinants from an *S. typhimurium* genomic library constructed in pBR322 were screened under conditions of moderate stringency with *E. coli umuDC* as a probe (55). Restriction enzyme analysis and nucleic acid hybridizations of the single positive clone identified in this screen indicated that this recombinant carried only a partial clone of the *S. typhimurium umuDC* operon. A 2.4-kb *Eco*RI fragment from this clone, containing the *S. typhimurium umu* sequences, was subsequently purified and used as a probe to screen a second *S. typhimurium* genomic library constructed in the lambda-dash vector. Positive clones (90 of 40,000) were identified in this second screen, and one was shown to contain both *umuD* and *umuC* by analysis of hybridizations to *E. coli umuDC* probes. A 4-kb *Eco*RI-*Bam*HI fragment containing the *S. typhimurium umuDC* operon was subcloned for functional characterization and sequence analysis.

Biological activity of the *S. typhimurium umuDC* operon. The 4-kb *Eco*RI-*Bam*HI fragment cloned into pBR322 restored mutability to *E. coli* mutants carrying either the *umuD44* or the *umuC36* allele (Fig. 1). Little enhancement of UV resistance (Fig. 1B), however, in either *umuC36* or *umuD44* (not shown) bacteria was observed. In contrast, the cloned *E. coli umuDC* operon enhanced both the mutability (Fig. 1, left panel) and UV resistance (Fig. 1, right panel) of both the *umuC36* and *umuD44* (not shown) strains. The degree of mutagenesis induced by UV in the presence of pSE117 was consistently higher (in terms of both absolute numbers of mutants and mutation frequencies) than that observed with the *S. typhimurium* clone. Weak restoration of mutability to *E. coli umuDC* mutants by an *S. typhimu-*

FIG. 2. The 2,497-nucleotide sequence of a region containing the *umuDC* locus from *S. typhimurium*. All but the 5'-terminal 41 bp and the 3'-terminal 304 bp were determined on both strands. Open reading frames beginning at positions 1 and 423 and their deduced amino acid sequences are shown. Termination codons are also denoted (‡). Putative regulatory regions are underlined. RBS, Possible ribosome-binding site. The 3' end of the sequence terminates at the previously identified *Eco*RI site (55, 58). CCGC denotes an ambiguous sequence.


-120
-100
-80
CCCATAATTTGAACTGAGAGAGAAACTTACAAACGAAGCGACGAAGATTTAAACAGTCGTAGCGACT
-60
-40
-20
CCGGTATCTTGCGCGCATGTTCAAATAACACTACTGTATATAAAAACAGTATTCGAGGTATGGATT
SOS BOX
RBS
1
ATGGAATTTTTCAGACCTACAGAGTTGCGCGAAATTATTCCTCTCCCATTTTTCAGTTACTTAGTGCCGTGTGGATTCCCCAGCCCC 87
M E F F R P T E L R E I I P L P F F S Y L V P C G F P S P
GCGGCGGACTACATTGAGCAGCGTATCGATCTTAATGAGTTGCTCGTTTCTCATCCCAGCTCAACATATTTTGTCAAAGCCTCGGGG 174
A A D Y I E Q R I D L N E L L V S H P S S T Y F V K A S G
GATTCAATGATTGAAGCAGGCATCAGCGACGGTGACCTGCTGGTGGTGGATAGCTCACGGAACGCTGACCACGGTGACATTGTAATT 261
D S M I E A G I S D G D L L V V D S S R N A D H G D I V I
GCGGCAATTGAAGGAGAGTTCACCGTAAAACGGTTGCAGTTGCGCCCGACAGTGCAGTTAATCCCCATGAACGGCGCCTATCGACCT 348
A A I E G E F T V K R L Q L R P T V Q L I P M N G A Y R P
ATACCTGTCGGCAGTGAAGACACGCTCGACATATTCGGGGTGGTGACCTTTATCATTAAAGCGGTCAGTTGATTATGTTCGCGCTCTGC 437
I P V G S E D T L D I F G V V T F I I K A V S ‡ M F A L C
GATGTTAATAGCTTTTACGCCTCCTGCGAAACGGTCTTTCGTCCTGATTTATGTGGCCGACCGGTGGTGGTGTTATCAAACAATGAT 524
D V N S F Y A S C E T V F R P D L C G R P V V V L S N N D
GGCTGCGTTATCGCGTGTAGCGCCGAGGCGAAACAGCTCGGTATCGCACCAGGTGAGCCATACTTCAAACAGAAAGAACGCTTCCGG 611
G C V I A C S A E A K Q L G I A P G E P Y F K Q K E R F R
CGATCCGGTGTTGTTTGCTTCAGCAGTAATTACGAGCTTTACGCTGATATGTCGAACCGGGTAATGACCACACTCGAGGAGATGGTG 698
R S G V V C F S S N Y E L Y A D M S N R V M T T L E E M V
CCGCGGGTAGAAATTTACAGCATTGATGAGGCCTTTTGTGATCTGACGGGGGTACGAAACTGCCGGGATCTGACAGATTTCGGGCGC 785
P R V E I Y S I D E A F C D L T G V R N C R D L T D F G R
GAGATAAGAGCGACGGTCCTGAAGCGCACGCACCTGACTGTCGGTGTAGGCATTGCCCAGACGAAAACCCTTGCCAAGCTGGCTAAC 872
E I R A T V L K R T H L T V G V G I A Q T K T L A K L A N
CATGCTGCGAAAAAGTGGCAGCGCCAGACCGACGGGGTGGTTGACTTGTCGAACATCGATCGCCAGCGTCGGCTGCTGGCCCTGATA 959
H A A K K W Q R Q T D G V V D L S N I D R Q R R L L A L I
CCCGTGGAGGATGTCTGGGGTGTCGGCAGGCGCATCAGTAAGAAGCTCAATGCCCTGGGCATCAAGACTGCTCTCGATCTCTCTGAA 1046
P V E D V W G V G R R I S K K L N A L G I K T A L D L S E
CAAAGTACCTGGATCATCAGGAAACACTTCAATGTCGTGCTGGAGCGTACCGTGAGAGAGCTTCGCGGAGAGCCATGTCTGGAGCTC 1133
Q S T W I I R K H F N V V L E R T V R E L R G E P C L E L
GAAGAGTTTGCGCCGGCAAAGCAGGAAATCGTTTGTAGTCGCTCTTTCGGCGAGCGGGTCACAGACTATGAGGAAATGCGCCAGGCT 1220
E E F A P A K Q E I V C S R S F G E R V T D Y E E M R Q A
GTTTACAGCTACGCTGCGCGCGCGGCAGAAAAACTCCGCGGCGAGCACCAGTACTGCCGTTTCATTTCAACATTCGTCAAAACATCA 1307
V Y S Y A A R A A E K L R G E H Q Y C R F I S T F V K T S
CCCTTTGCCCTGAACGAGCCCTACTACGGTAACAGCGCCGCGGTGACGCTTCTCACCCCCACGCAGGATTCACGTGACATTATCAAT 1394
P F A L N E P Y Y G N S A A V T L L T P T Q D S R D I I N
GCGGCTGTGAAATGCCTGGATAAAATATGGCGCGACGGCCATCGCTACCAGAAAGCGGGGGTGATGCTGGGTGACTTCTTCAGTCAG 1481
A A V K C L D K I W R D G H R Y Q K A G V M L G D F F S Q
GGCGTAGCGCAACTCAACCTTTTCGACGATAACGCGCCGCGCGCCGGTAGTGCGAAGTTGATGGAAGTACTGGACCATCTTAACGCA 1568
G V A Q L N L F D D N A P R A G S A K L M E V L D H L N A
AAAGACGGGAAGGGGACGCTGTACTTCGCCGGGCAGGGGATGTCGCAACAGTGGGCTATGAAGCGAGAAATGCTTTCGCCTCGGTAC 1655
K D G K G T L Y F A G Q G M S Q Q W A M K R E M L S P R Y
ACCACAAGATACTCTGATCTACTGCGTGTTAAGTAACTTGTGCGATCAATGCCTGAGATGGTTGCCAAATCATCCCCGTTCTCTAAC 1742
T T R Y S D L L R V K ‡
CGGTTTTGGTCGCACAAGATCACAGGAACCTCTCACGATGAGCTTGTGCGATCAATGCCTGAGATGGTTGCCAAATCATCCCCGTTCTCTAACCGGTTTTGGTCGCA
CAAGATCACAGGAACCTCTCACGATGAGGCGCATGTATCCTGGTTTACGACATCAGAAAATGTGGCGCGTTTATTGCCCGGCAGGCGTTGTGAGACGTCACTTATTT
ACGCCAGGTTTCAGCCGTAGCGACAGGCATGAATAAAAAGAGTATGGCAATCAGCGTGATAATGCTAAAAAACAATTAATATTTTTTTAACAAAACTAAAGCTTGCT
ATGTTCAGTTAACCATGCGTTAATGGTTGTGCGGTTTGATACAAACTTATCTGAAGTAGTGATTGTAATATTTCTCATCATTTGTTCCTCTTGAGATCTCCTTTAGG
TTTTTTTCTCTCTGATAATTTTCTTCAGGCCATTTTCCGCAAGGGCTCATTCGAAAGGTAACAATATTATGACGACGAAAATCACTGGTTTAGTAAAATGGTTTAAC
CCTGAAAAGGGCTTTGGTTTCATTACGCCTAAAGATGGCAGCAAAGATGTGTTTGTGCATTTTTCAGCCATTCAAAGTAATGAATTC
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rium umuDC clone was also reported by Thomas and Sedgwick (58).

Sequence analysis. (i) Nucleotide sequence comparisons. Sequencing reactions were performed on DNA fragments subcloned into M13 and pBR322 and on chromosomal fragments that had been asymmetrically amplified by the polymerase chain reaction. The nucleotide sequence of 2,497 bp that covered a portion of the clone proximal to the *Eco*RI site is displayed in Fig. 2.

The sequence contained two nonoverlapping open reading frames of 417 and 1,266 bp encoding two proteins with calculated molecular sizes of 15,289 and 47,833 daltons, respectively. Nucleotide sequences were compared with the sequences of *umuDC* from *E. coli* and *mucAB* with the aid of the Wilbur-Lipman algorithm (67). Alignments were performed separately for the upstream regulatory regions and for each member of the operon. Sequence comparisons of the coding regions for the two chromosomal *umu* operons are presented in Fig. 3.

The *S. typhimurium umuD* and *umuC* coding sequences were 67 and 72% identical, respectively, to their cognate sequences in *E. coli*. The degree of similarity between the *S. typhimurium umuD* and *umuC* coding sequences and their *muc* operon counterparts was less than 50% for each gene and corresponded to the degree of similarity between the *E. coli umu* operon and the *muc* operon. Overall G+C content of the *S. typhimurium umuDC* operon was 54% (62% at the third codon position) compared with 50% in *E. coli* (53% at the third codon position). *S. typhimurium* typically has a higher G+C content than *E. coli* (43). A comparison of codon usage for the two *umuDC* operons is summarized in Table 2. The comparison reveals that codon usage for the two operons was similar in the two organisms for all but a few codons (e.g., Phe and Tyr in *umuC*). A codon usage analysis by Paul Sharp et al. revealed that both *umuDC* operons have low frequencies of optimal codons, a characteristic of genes that are not expressed at high levels (50; P. Sharp, personal communication).

Sharp has also estimated the nucleotide sequence divergence at silent sites of the *E. coli* and *S. typhimurium umuDC* operons (personal communication) and found that the divergence was high. The degree of divergence at silent sites among 67 previously analyzed pairs of *E. coli* and *S. typhimurium* genes (50) was related to the codon usage bias (a measure of optimal codon usage) and, among genes with nonoptimal codon usage, to chromosomal location (genes furthest from *oriC* were, on average, approximately twice as divergent as genes near *oriC*; 50). Compared with the 67 *E. coli* and *S. typhimurium* genes analyzed by Sharp, *umuD* was the most divergent, and only three genes (*pabB*, *sulA*, and *tar*) were more divergent than *umuC*. The degree of divergence is consistent with the low usage of optimal codons by the *umuDC* operon and with its chromosome position far from *oriC* (see below).

In *S. typhimurium* the coding regions for *umuD* and *umuC* were separated by a TT dinucleotide. This contrasted with the overlapping reading frames found in *E. coli* (1 bp; 21, 40) and *muc* (13 bp; 40). Nucleotide sequence divergence was highest in the region encoding the N-terminal portion of *umuD*; nucleotide sequence similarity for the portion encoding the first 24 amino acids (i.e., up to the Cys-24–Gly-25 cleavage site) was 62%; for the portion encoding the remaining 115 amino acids, similarity was 73%.

A canonical 16-bp SOS box was identified beginning 18 bp upstream of *umuD* (66). The sequence of the SOS box in *S. typhimurium* was identical to that of the SOS box upstream of *umuD* in *E. coli* but was located 4 bp closer to the initiation codon. In *E. coli* there is a second but apparently nonfunctional SOS box beginning 4 bp upstream from the first box (21); this sequence was not present in *S. typhimurium*. Potential ribosome-binding sites were found just upstream of the initiation codons for *umuD* (..tcGAGGTat..) and *umuC* (..aaGcGGTcAgt..).

The nucleotide sequence between the SOS boxes and the *umuD* start codon could be aligned with the *E. coli* sequence to yield 65% identity (11 of 17) when a single base gap in the *S. typhimurium* sequence was allowed. Considerable sequence diversity upstream of the SOS boxes obtained among the two chromosomal *umu* operons and the two plasmid-borne operons, *muc* and *imp*. In the −35 region of each operon, however, a conserved TTGNNNNNNTG motif was observed (Fig. 4). This motif has features in common with −35 regions from other members of the SOS regulon (18) and probably represents promoter recognition elements. The functional significance of the variability in these regulatory regions is not known.

(ii) Amino acid sequence comparisons. Amino acid sequences were compared with the sequences of UmuDC from *E. coli* and MucAB with the aid of the Needleman-Wunsch algorithms (35). Amino acid sequence comparisons among the two UmuD proteins and MucA on the one hand and the two UmuC proteins and MucB on the other hand are presented in Fig. 5 and summarized in Table 3. Amino acid sequence identity between the Umu proteins was 73% for UmuD and 84% for UmuC. If evolutionarily conservative amino acid substitutions were taken into account, the similarity was 90% for UmuD and 93% for UmuC. The N-terminal portions of the UmuD proteins, up to the Cys-24–Gly-25 cleavage site, displayed only 50% sequence identity (79% similarity including conservative substitutions). The calculated isoelectric points indicate that, similar to *E. coli* UmuC, *S. typhimurium* UmuC will be basic and have the potential to bind to DNA and to UmuD (40, 68).

Similarity between Umu proteins and their Muc homologs was considerably less. Between UmuD from *S. typhimurium* and MucA there was only 42% identity; between UmuC and MucB there was only 54% identity. Perry et al. (40) found that sequence identity was 41% between *E. coli* UmuD and MucA and 55% between *E. coli* UmuC and MucB. Thus, the bacterial Umu products are more similar to one another than they are to the plasmid-encoded Muc products.

The UmuD protein from *S. typhimurium* retained primary structural features thought to be important components of RecA-enhanced posttranslational processing: in common with *E. coli* UmuD, *S. typhimurium* UmuD had a proteolytic cleavage site (Cys-24–Gly-25) and the Ser-60 and Lys-97 residues believed to play a catalytic role in autoproteolytic cleavage (15, 24, 26, 27, 36, 54).

Mapping *umuDC* with locked-in Mu d-P22 prophages. We assumed that the chromosomal location of *umuDC* in *S. typhimurium* would be similar to its location in *E. coli* at 26 min (1). Bearing in mind, however, that the order of genes in

FIG. 3. Nucleotide and amino acid sequences of *S. typhimurium* (ST) and *E. coli* (EC) *umuDC*. The complete coding sequences from *S. typhimurium* are given, and the differences in the *E. coli* sequences are indicated below the *S. typhimurium* sequence. ● (at Cys-24–Gly-25 junction of UmuD), Putative cleavage site.

```
      1                                                                  18
ST  MET Glu Phe Phe Arg Pro Thr Glu Leu Arg Glu Ile Ile Pro Leu Pro Phe Phe
    ATG GAA TTT TTC AGA CCT ACA GAG TTG CGC GAA ATT ATT CCT CTC CCA TTT TTC
        TTG     A    AG     G G   T C C             G G A   T T   G C A   T
EC      Leu     Ile Lys     Ala Asp                 Val Thr Phe     Leu

                                                                       36
Ser Tyr Leu Val Pro Cys•Gly Phe Pro Ser Pro Ala Ala Asp Tyr Ile Glu Gln
AGT TAC TTA GTG CCG TGT GGA TTC CCC AGC CCC GCG GCG GAC TAC ATT GAG CAG
  C G T C T   T  A       C   T   T TCA   G   A   A   T     G     A
    Asp         Gln                                         Val

                                                                       54
Arg Ile Asp Leu Asn Glu Leu Leu Val Ser His Pro Ser Ser Thr Tyr Phe Val
CGT ATC GAT CTT AAT GAG TTG CTC GTT TCT CAT CCC AGC TCA ACA TAT TTT GTC
  C           G     C A C   T G A C CAG             G G   T   C   C
                    Gln         Ile Gln             Ala

                                                                       72
Lys Ala Ser Gly Asp Ser Met Ile Glu Ala Gly Ile Ser Asp Gly Asp Leu Leu
AAA GCC TCG GGG GAT TCA ATG ATT GAA GCA GGC ATC AGC GAC GGT GAC CTG CTG
      A AGT   T       T           T  GT   A   T   T           T T A
                                  Asp Gly

                                                                       90
Val Val Asp Ser Ser Arg Asn Ala Asp His Gly Asp Ile Val Ile Ala Ala Ile
GTG GTG GAT AGC TCA CGG AAC GCT GAC CAC GGT GAC ATT GTA ATT GCG GCA ATT
A T   C         G T ATT  C    C AG    T       T       C   C   T   T G
Ile             Ala Ile Thr     Ser                                 Val

                                                                      108
Glu Gly Glu Phe Thr Val Lys Arg Leu Gln Leu Arg Pro Thr Val Gln Leu Ile
GAA GGA GAG TTC ACC GTA AAA CGG TTG CAG TTG CGC CCG ACA GTG CAG TTA ATC
  C   C       T   G   G     AAA       A C A           G   A     C T   T
Asp                         Lys

                                                                      126
Pro Met Asn Gly Ala Tyr Arg Pro Ile Pro Val Gly Ser Glu Asp Thr Leu Asp
CCC ATG AAC GGC GCC TAT CGA CCT ATA CCT GTC GGC AGT GAA GAC ACG CTC GAC
            A     G   C TCG   C   T A C A   A T           T       G   T
            Ser         Ser         Thr Ile Ser

                                                  139         1
Ile Phe Gly Val Val Thr Phe Ile Ile Lys Ala Val Ser END   MET
ATA TTC GGG GTG GTG ACC TTT ATC ATT AAA GCG GTC AGT TGATT ATG
G C   T   T           T  CAC G   G     G     A G C C     G
Val                 Ile His Val Val         Met Arg   MET ---
                                                      1
  2                                                          18
Phe Ala Leu Cys Asp Val Asn Ser Phe Tyr Ala Ser Cys Glu Thr Val Phe
TTC GCG CTC TGC GAT GTT AAT AGC TTT TAC GCC TCC TGC GAA ACG GTC TTT
  T   C       T       A   C GCG       T     AG    T   G       G
                            Ala

                                                                       36
Arg Pro Asp Leu Cys Gly Arg Pro Val Val Val Leu Ser Asn Asn Asp Gly Cys
CGT CCT GAT TTA TGT GGC CGA CCG GTG GTG GTG TTA TCA AAC AAT GAT GGC TGC
  C               G   T AA           T     C     G   T       C   T
                Trp     Lys

                                                                       54
Val Ile Ala Cys Ser Ala Glu Ala Lys Gln Leu Gly Ile Ala Pro Gly Glu Pro
GTT ATC GCG TGT AGC GCC GAG GCG AAA CAG CTC GGT ATC GCA CCA GGT GAG CCA
          C C A  A    T       A   G GC    T   C G T AA  ATG   C   T   C
            Arg Asn             Ala         Val Lys Met     Asp
```

```
                                                                      72
Tyr Phe Lys Gln Lys Glu Arg Phe Arg Arg Ser Gly Val Val Cys Phe Ser Ser
TAC TTC AAA CAG AAA GAA CGC TTC CGG CGA TCC GGT GTT GTT TGC TTC AGC AGT
 GG           A       T  TG   T   T   C  GT   C   G           T       C
Trp                 Asp Leu             Cys

                                                                      90
Asn Tyr Glu Leu Tyr Ala Asp Met Ser Asn Arg Val Met Thr Thr Leu Glu Glu
AAT TAC GAG CTT TAC GCT GAT ATG TCG AAC CGG GTA ATG ACC ACA CTC GAG GAG
      T               A   C     AGC   T       G     T G   G   G   A
                                                    Ser

                                                                     108
Met Val Pro Arg Val Glu Ile Tyr Ser Ile Asp Glu Ala Phe Cys Asp Leu Thr
ATG GTG CCG CGG GTA GAA ATT TAC AGC ATT GAT GAG GCC TTT TGT GAT CTG ACG
C A TC    C   C   C   G           T               A   C   C           A
Leu Ser

                                                                     126
Gly Val Arg Asn Cys Arg Asp Leu Thr Asp Phe Gly Arg Glu Ile Arg Ala Thr
GGG GTA CGA AAC TGC CGG GAT CTG ACA GAT TTC GGG CGC GAG ATA AGA GCG ACG
  T   G   T   T   T   C           T       T   C A A   A   T C C   A

                                                                     144
Val Leu Lys Arg Thr His Leu Thr Val Gly Val Gly Ile Ala Gln Thr Lys Thr
GTC CTG AAG CGC ACG CAC CTG ACT GTC GGT GTA GGC ATT GCC CAG ACG AAA ACC
  G   A C A   T   C   T   T       T       G   G   C           C       G
        Gln

                                                                     162
Leu Ala Lys Leu Ala Asn His Ala Ala Lys Lys Trp Gln Arg Gln Thr Asp Gly
CTT GCC AAG CTG GCT AAC CAT GCT GCG AAA AAG TGG CAG CGC CAG ACC GAC GGG
  G   T       T   C   T       G   A       A           G       G  GT
                                                                Gly

                                                                     180
Val Val Asp Leu Ser Asn Ile Asp Arg Gln Arg Arg Leu Leu Ala Leu Ile Pro
GTG GTT GAC TTG TCG AAC ATC GAT CGC CAG CGT CGG CTG CTG GCC CTG ATA CCC
  G   G   T   A   A   T C G   A             AAA T A A   T T GCT C C
                      Leu Glu             Lys     Met Ser Ala Leu

                                                                     198
Val Glu Asp Val Trp Gly Val Gly Arg Arg Ile Ser Lys Lys Leu Asn Ala Leu
GTG GAG GAT GTC TGG GGT GTC GGC AGG CGC ATC AGT AAG AAG CTC AAT GCC CTG
      T   C           G A T   A C     G       C   A   A   G G C   G A
    Asp                 Ile                             Asp     Met

                                                                     216
Gly Ile Lys Thr Ala Leu Asp Leu Ser Glu Gln Ser Thr Trp Ile Ile Arg Lys
GGC ATC AAG ACT GCT CTC GAT CTC TCT GAA CAA AGT ACC TGG ATC ATC AGG AAA
  G       A   C  T           T G G G   T AC  GA   T  C   T T     C T
               Val             Ala Asp Thr Asp Ile Arg Phe

                                                                     234
His Phe Asn Val Val Leu Glu Arg Thr Val Arg Glu Leu Arg Gly Glu Pro Cys
CAC TTC AAT GTC GTG CTG GAG CGT ACC GTG AGA GAG CTT CGC GGA GAG CCA TGT
  T   T               C   A A A   G     C T   A   G       C   A   C
```

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                                                                    252
Leu Glu Leu Glu Glu Phe Ala Pro Ala Lys Gln Glu Ile Val Cys Ser Arg Ser
CTG GAG CTC GAA GAG TTT GCG CCG GCA AAG CAG GAA ATC GTT TGT AGT CGC TCT
T G C A   G               A     A G               T A C     TCC       G
                                  Thr                 Ile

                                                                    270
Phe Gly Glu Arg Val Thr Asp Tyr Glu Glu Met Arg Gln Ala Val Tyr Ser Tyr
TTC GGC GAG CGG GTC ACA GAC TAT GAG GAA ATG CGC CAG GCT GTT TAC AGC TAC
  T   T   A   C A     G   T     CC  TCG       G       C A    GT   T
                Ile             Pro Ser                 Ile Cys

                                                                    288
Ala Ala Arg Ala Ala Glu Lys Leu Arg Gly Glu His Gln Tyr Cys Arg Phe Ile
GCT GCG CGC GCG GCA GAA AAA CTC CGC GGC GAG CAC CAG TAC TGC CGT TTC ATT
      C   G       G           T     A         T   A   T   T   G   T   C
                                    Ser

                                                                    306
Ser Thr Phe Val Lys Thr Ser Pro Phe Ala Leu Asn Glu Pro Tyr Tyr Gly Asn
TCA ACA TTC GTC AAA ACA TCA CCC TTT GCC CTG AAC GAG CCC TAC TAC GGT AAC
  C   G   T A T   G   G       A       G   C   T   A   T   T       C   T
            Ile

                                                                    324
Ser Ala Ala Val Thr Leu Leu Thr Pro Thr Gln Asp Ser Arg Asp Ile Ile Asn
AGC GCC GCG GTG ACG CTT CTC ACC CCC ACG CAG GAT TCA CGT GAC ATT ATC AAT
      G T     A  AA   G   G   G       T       C AGC A G   T   C   T   C
        Ser     Lys

                                                                    342
Ala Ala Val Lys Cys Leu Asp Lys Ile Trp Arg Asp Gly His Arg Tyr Gln Lys
GCG GCT GTG AAA TGC CTG GAT AAA ATA TGG CGC GAC GGC CAT CGC TAC CAG AAA
  T     AC  CG   CT         GCC   C      AA  CG           T       A
        Thr Arg Ser         Ala         Gln Ala

                                                                    360
Ala Gly Val Met Leu Gly Asp Phe Phe Ser Gln Gly Val Ala Gln Leu Asn Leu
GCG GGG GTG ATG CTG GGT GAC TTC TTC AGT CAG GGC GTA GCG CAA CTC AAC CTT
      C               G   T                   A   C       G       T T A

                                                                    378
Phe Asp Asp Asn Ala Pro Arg Ala Gly Ser Ala Lys Leu Met Glu Val Leu Asp
TTC GAC GAT AAC GCG CCG CGC GCC GGT AGT GCG AAG TTG ATG GAA GTA CTG GAC
      T   C       A         C     G      A  C A         ACG     A     T
                            Pro         Glu Gln         Thr     Met

                                                                    396
His Leu Asn Ala Lys Asp Gly Lys Gly Thr Leu Tyr Phe Ala Gly Gln Gly Met
CAT CTT AAC GCA AAA GAC GGG AAG GGG ACG CTG TAC TTC GCC GGG CAG GGG ATG
ACA   G   T   T       G   C  GA   A   A   C   T   T                   C
Thr                 Glu     Arg                                        Ile

                                                                    414
Ser Gln Gln Trp Ala Met Lys Arg Glu Met Leu Ser Pro Arg Tyr Thr Thr Arg
TCG CAA CAG TGG GCT ATG AAG CGA GAA ATG CTT TCG CCT CGG TAC ACC ACA AGA
CA        A     CAG             CC          A   A   T   T   A   G C
Gln             Gln             Ala

                                422
Tyr Ser Asp Leu Leu Arg Val Lys END
TAC TCT GAT CTA CTG CGT GTT AAG TAA
AGT         T       A G   C   A
Ser
```

TABLE 2. Codon usage

Protein	Codon	No. of times used[a]		Codon	No. of times used		Codon	No. of times used		Codon	No. of times used	
		ST	EC		ST	EC		ST	EC		ST	EC
UmuD	TTT	4	6	TCT	1	1	TAT	2	0	TGT	1	1
	TTC	5	1	TCC	0	0	TAC	2	3	TGC	0	0
	TTA	2	1	TCA	3	1	TAA	0	0	TGA	1	1
	TTG	4	3	TCG	1	1	TAG	0	0	TGG	0	0
	CTT	1	2	CCT	4	2	CAT	1	2	CGT	1	0
	CTC	3	1	CCC	4	3	CAC	1	1	CGC	2	4
	CTA	0	2	CCA	1	0	CAA	0	2	CGA	1	0
	CTG	2	4	CCG	2	3	CAG	3	4	CGG	2	0
	ATT	8	8	ACT	0	2	AAT	1	1	AGT	3	4
	ATC	4	6	ACC	2	2	AAC	2	1	AGC	4	5
	ATA	2	0	ACA	3	0	AAA	3	3	AGA	1	0
	ATG	3	4	ACG	1	3	AAG	0	2	AGG	0	0
	GTT	1	4	GCT	1	3	GAT	3	11	GGT	2	5
	GTC	3	5	GCC	2	1	GAC	7	2	GGC	3	2
	GTA	2	1	GCA	2	3	GAA	5	3	GGA	2	1
	GTG	6	4	GCG	4	4	GAG	4	1	GGG	2	0
UmuC	TTT	5	14	TCT	3	3	TAT	1	7	TGT	5	8
	TTC	13	5	TCC	2	2	TAC	14	5	TGC	7	3
	TTA	2	5	TCA	4	3	TAA	1	1	TGA	0	0
	TTG	2	3	TCG	4	6	TAG	0	0	TGG	5	6
	CTT	7	6	CCT	2	2	CAT	3	5	CGT	6	8
	CTC	10	7	CCC	4	6	CAC	3	0	CGC	14	11
	CTA	1	3	CCA	3	2	CAA	3	9	CGA	4	4
	CTG	17	15	CCG	4	4	CAG	14	12	CGG	8	8
	ATT	5	8	ACT	2	3	AAT	6	12	AGT	6	5
	ATC	9	12	ACC	7	3	AAC	10	4	AGC	7	8
	ATA	3	0	ACA	6	5	AAA	12	17	AGA	3	3
	ATG	10	12	ACG	8	14	AAG	11	5	AGG	2	2
	GTT	8	6	GCT	8	7	GAT	15	20	GGT	8	6
	GTC	8	5	GCC	10	10	GAC	9	6	GGC	9	9
	GTA	6	3	GCA	4	7	GAA	10	11	GGA	1	3
	GTG	11	14	GCG	13	12	GAG	17	10	GGG	8	8

[a] ST, *S. typhimurium*; EC, *E. coli*.

the 25- to 35-min region of the *S. typhimurium* chromosome is inverted with respect to the order in *E. coli* (1, 43, 45; Fig. 6), we searched both ends of the inverted region for sequences that hybridized to *umu* probes.

To obtain chromosomal DNA from the desired regions, we used Mu d-P22 prophages inserted at either end of the inversion. Mu d-P22 prophages are hybrid genetic elements consisting of a defective P22 genome sandwiched between the ends of transposon Mu (70). Insertion of Mu d-P22 in the *Salmonella* chromosome generates a locked-in prophage that cannot excise. When P22 is induced by DNA-damaging treatments, replication of P22 DNA occurs in situ, leading to amplification of neighboring regions of the bacterial chromosome. Packaging of amplified DNA by P22 phage heads preferentially starts from the P22 *pac* site. The directionality of packaging enriches for chromosomal DNA sequences that are downstream from a *pac* site. As the packaging specificity is not absolute, however, chromosomal sequences upstream from the *pac* site can also be represented in the phage lysates, although to a much lesser extent than the down-

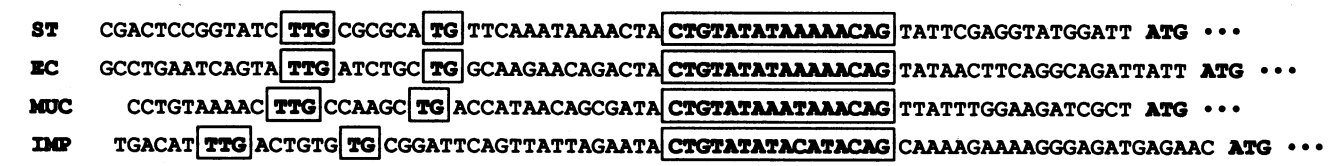

FIG. 4. Comparison of regulatory regions upstream from *umuDC* operons of *S. typhimurium* (ST) and *E. coli* (EC) and from plasmid-borne operons *mucAB* (MUC) and *impAB* (IMP). The *lexA* boxes from each of these operons have been aligned and boxed. Boxes to the left highlight the TTGNNNNNNTG motif common to all four operons.

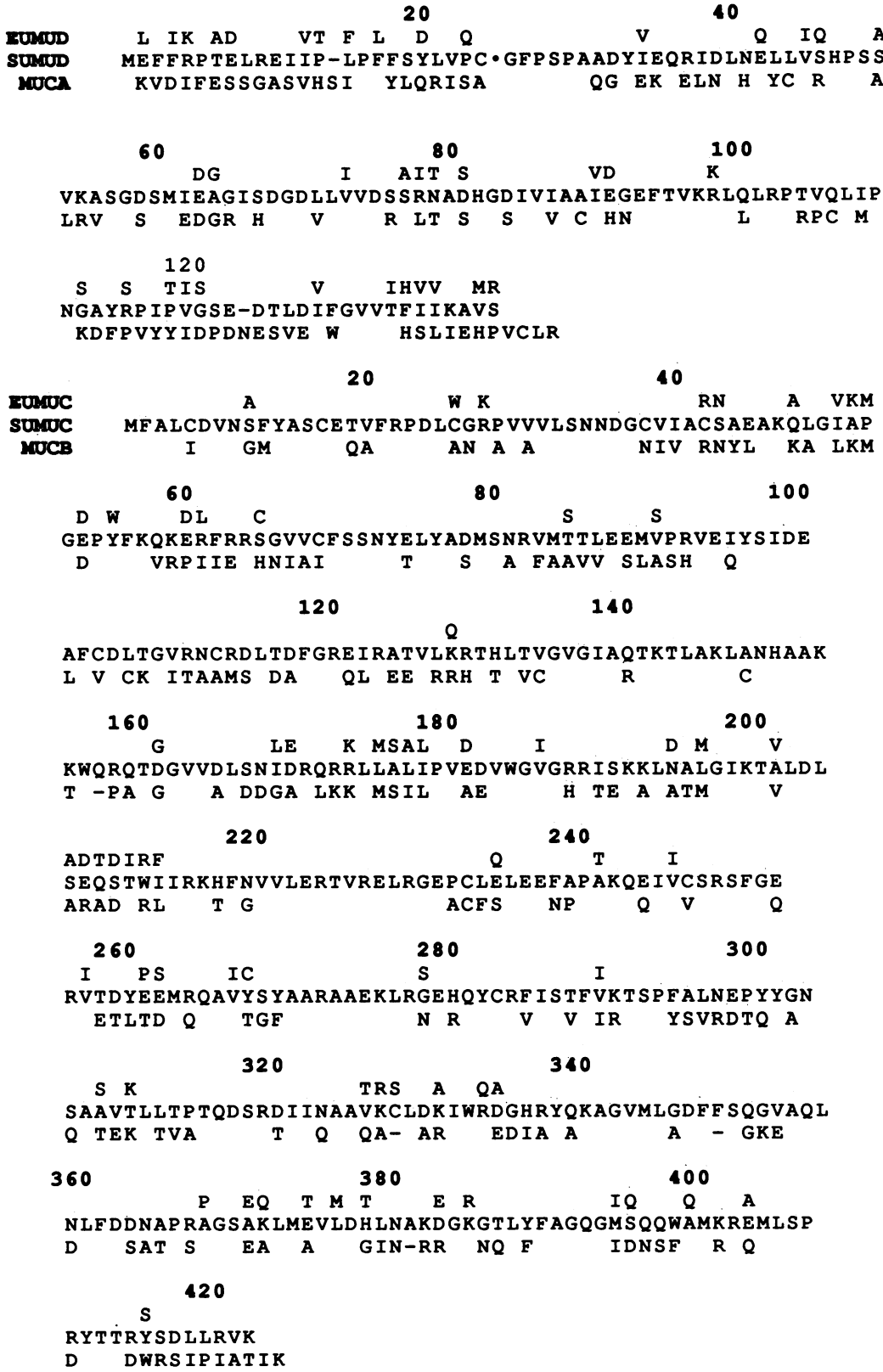

FIG. 5. Amino acid sequences of UmuD and UmuC from *S. typhimurium* (SUMUD and SUMUC) and *E. coli* (EUMUD and EUMUC) and of MucA and MucB. The complete amino acid sequence of the *S. typhimurium* products is shown, with differences from *E. coli* and Muc proteins indicated above and below, respectively.

TABLE 3. Deduced amino acid compositions of *umu* and *muc* gene products[a]

Amino acid	ST *umuD*	EC *umuD*	*mucA*	ST *umuC*	EC *umuC*	*mucB*
Ala	9	11	6	35	36	56
Arg	7	4	9	37	36	35
Asn	3	2	4	16	16	15
Asp	10	13	8	24	26	22
Cys	1	1	4	12	11	6
Gln	3	6	3	17	21	18
Glu	9	4	9	27	21	21
Gly	9	8	8	26	26	25
His	2	3	8	6	5	5
Ile	14	14	9	17	20	24
Leu	12	13	12	39	39	35
Lys	3	5	4	23	22	20
Met	3	4	4	10	12	12
Phe	9	7	6	18	19	17
Pro	11	8	10	13	14	11
Ser	12	12	15	26	27	25
Thr	6	7	4	23	25	27
Trp	0	0	1	5	6	4
Tyr	4	3	6	15	12	11
Val	12	14	15	33	28	31
Mol wt	15,289	15,064	16,372	47,833	47,684	46,366
Isoelectric point	4.3	4.3	6.3	8.6	8.9	9.4

[a] ST, *S. typhimurium*; EC, *E. coli*.

stream sequences (70). Chromosomal Mu dJ insertions can be converted to Mu d-P22 inserts by recombination between the ends of Mu. Thus, one can preferentially package chromosomal DNA on either side of a given Mu dJ insertion by converting it to each of the two available orientations of Mu d-P22.

Phage lysates from strains carrying both packaging orientations of Mu d-P22 inserted near 25, 37, and 40 min were prepared as described in Materials and Methods and probed with oligonucleotides specific for *umuDC* and for genes known to map in these regions. For the 25-min region, we used a probe for *phoP* (31), and for the 37- to 40-min region, we used a probe for *dadB* (65). We also used a probe for the *icd* locus (59), which is located approximately 38 kb counterclockwise from *umuDC* in *E. coli* (17; K. E. Rudd, personal communication). The *S. typhimurium icd* gene had not previously been mapped or characterized.

Both the *phoP* and *icd* probes hybridized preferentially to lysates packaging clockwise from a Mu d-P22 insertion at 25 min; the *dadB* probe hybridized preferentially to lysates packaging clockwise from a Mu d-P22 insertion at 35.9 min and counterclockwise from a Mu d-P22 insertion at 40.5 min (Fig. 7). The *umuC*-specific probe hybridized most strongly to the insertions packaging clockwise from 35.9 min and counterclockwise from 40.5 min, thus indicating that the *umuDC* locus was located between the two insertions. Identical results were obtained when DNA from the phage lysates was extracted and probed. Although P22 packaging occurs preferentially downstream from the *pac* site, some amplification of upstream sequence can also occur (70). This accounts for the presence of weaker hybridization signals observed counterclockwise of 35.9 min and clockwise of 40.5 min with the *umuC* and *dadB* probes (Fig. 7).

To confirm the identity of the DNA material detected in the dot blots by the *umuC* probe, we performed a Southern hybridization analysis on DNA samples digested with both

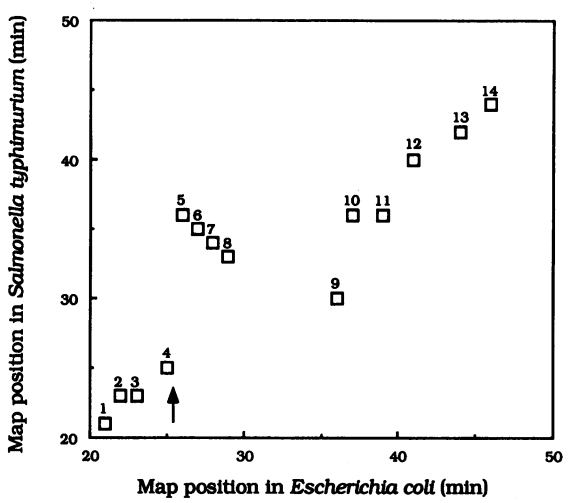

FIG. 6. Map positions of genes in the 20- to 45-min region of the chromosomes from *S. typhimurium* and *E. coli*. The departure from linearity marked by the arrow at 25 min and extending to about 35 min identifies the region in which gene order is inverted on one chromosome with respect to the other. Map positions rounded off to the nearest minute were obtained from references 1 and 45. Numbers refer to the following loci: 1, *pyrD*; 2, *put*; 3, *pyrC*; 4, *purB*; 5, *tre*; 6, *galU*; 7, *trp*; 8, *tyrR*; 9, *pmi* in *S. typhimurium* (=*manA* in *E. coli*); 10, *pncA*; 11, *aroD*; 12, *cheZ*; 13, *his*; 14, *metG*.

*Eco*RI and *Bam*HI. The results show that the predicted (55) 4-kb fragment was enriched in the Mu dP-22 lysates packaging clockwise from 35.9 min and counterclockwise from 40.5 min (Fig. 8).

We conclude that the *umuDC* operon in *S. typhimurium* is located 10 to 15 min clockwise of its position in *E. coli*, thus corresponding to one end of an inversion breakpoint that lies between the *icd* and *umuDC* loci in *E. coli*.

DISCUSSION

Nucleotide sequence comparisons and evolutionary considerations. A difference between the two bacterial operons is

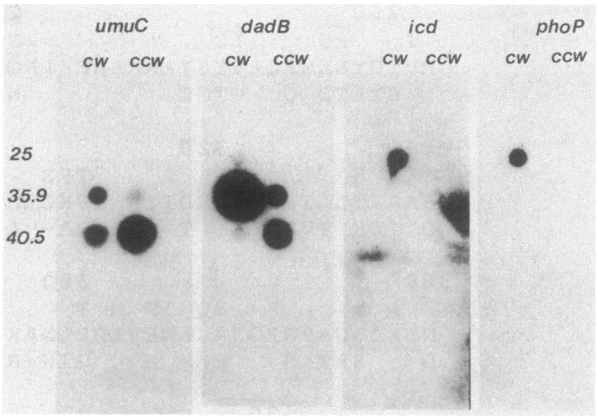

FIG. 7. Chromosomal location of *umuDC* in *S. typhimurium*. Dot blots of phage lysates (80 μl; 70) from strains carrying Mu d-P22 insertions in each orientation at 25, 35.9, and 40.5 min were vacuum blotted, dried, and probed with oligonucleotides specific for *umuC*, *dadB*, *icd*, or *phoP*. Numbers to the left identify the map positions of the Mu d-P22 inserts. Probes are identified at the top of each panel. The packaging of the Mu d-P22 inserts is identified as clockwise (cw) or counterclockwise (ccw).

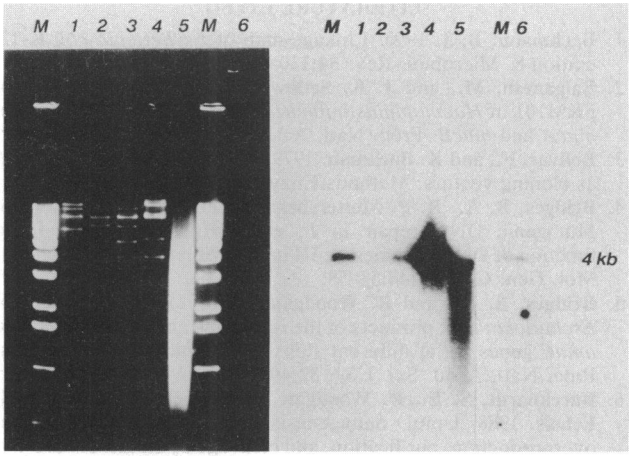

FIG. 8. Southern hybridization analysis of DNA from Mu d-P22 lysates probed with *S. typhimurium umuC*-specific oligonucleotide. DNA was harvested (70) from Mu d-P22 inserts packaging in both orientations at 35.9 and 40.5 min. Left, Ethidium bromide-stained agarose gel (0.8%) after electrophoresis of DNA samples that had been cut with *Eco*RI and *Bam*HI. Right, Autoradiographs obtained following blotting, hybridization, and washing. Lanes 1 to 4, Mu d-P22 DNA samples (5 μg each); lanes 1, 35.9 min, clockwise; lanes 2, 35.9 min, counterclockwise; lanes 3, 40.5 min, clockwise; lanes 4, 40.5 min, counterclockwise; lanes 5, *S. typhimurium* chromosomal DNA sample (5 μg); lanes 6, pSTumu (1 ng); lanes M, 1-kb DNA ladder molecular weight markers from Bethesda Research Laboratories, Inc., Gaithersburg, Md.

the organization of the region between the *umuD* and *umuC* genes. In *E. coli*, *umuD* and *umuC* overlap by a single nucleotide, whereas in *S. typhimurium*, a TT dinucleotide separates the *umuD* stop codon from the *umuC* initiation codon. The *mucA* and *mucB* loci overlap by 13 bp. The extent of overlap between contiguous members of an operon presumably influences translational coupling between them (69), but the functional significance of these intergenic sequence variations remains to be explored for the *umuDC* and *mucAB* operon families.

Although there was a relatively high degree of nucleotide sequence divergence at silent sites, the bacterial *umuDC* sequences were much more similar to each other than they were to that of the plasmid-borne *mucAB*. Limited sequence data (corresponding to UmuC amino acid residues 308 to 366) for 59 amino acid residues of ImpB (56) also indicate a low degree of similarity (35 of 59 identities between either of the two bacterial UmuC sequences and ImpB and 32 of 59 identities between ImpB and MucB sequences, but 50 of 59 identities between the two bacterial UmuC sequences). Across this limited stretch, the two plasmid-encoded proteins do not appear to be more similar to each other than to the bacterial UmuC proteins. This comparison further emphasizes the similarity of the bacterial loci.

Sedgwick et al. (49) observed that restriction fragment polymorphisms surround the *umuDC* region among different *Escherichia* isolates and identified sequences bracketing *umuDC* that resemble the termini of Tn*3*-related transposons. These observations lent support to suggestions, based on the widespread distribution of *umu*-like sequences on extrachromosomal elements, that *umuDC* was introduced into the *E. coli* lineage via transposition or replicon fusion (2, 49). It was imaginable, therefore, that the *E. coli* and *S. typhimurium* versions of *umuDC* were derived independently by horizontal transmission. Had this been the case, the chromosomal map positions of the *E. coli* and *S. typhimurium umuDC* operons might have been unrelated.

Our results localized the *S. typhimurium umuDC* operon to a site between 35.9 and 40 min, very near the end of a region whose gene order is inverted in *S. typhimurium* with respect to the order in *E. coli*. In *E. coli*, the *icd* locus is approximately 38 kb counterclockwise of the *umuDC* locus (Rudd, personal communication; 22, 44). The physical mapping data presented here show that *icd* (near 25 min) and *umuDC* (near 35 min) are separated by approximately 450 kb (1 min ≈ 45 kb; 45), which suggests that one end of the inversion breakpoint lies within the 38-kb region between *icd* and *umuDC*. Until higher-resolution mapping data are obtained, we cannot rule out the unlikely possibility that *S. typhimurium umuDC* lies outside the inverted region.

The mapping data support the notion that the *umuDC* operon was present in a common ancestor prior to the evolutionary divergence of *E. coli* and *S. typhimurium* approximately 150 million years ago (37). Mapping and nucleotide sequence data for other *umuDC* homologs might be useful for evaluating phylogenetic relationships among enterobacteria. Divergence within the highly variable N-terminal portion of *umuD* may also be of particular value as a measure of phylogenetic distance.

Structure-function considerations. Perry et al. (40) observed that the UmuD and MucA proteins were homologous to LexA. Key primary structural features preserved among members of this protein family are the amino residues corresponding to Cys (or Ala)-24–Gly-25, Ser-60, and Lys-97. These residues are now believed to be important for the RecA-mediated posttranslational modification of UmuD: the Cys-24–Gly-25 junction defines the cleavage site, while the Ser-60 and Lys-97 residues play catalytic roles in the cleavage event (15, 24, 36, 54). Lys-97 may also be critical for mutability functions (36) of UmuD′. These primary structural features were all preserved in *S. typhimurium* UmuD and strongly suggest that it, too, is activatable to UmuD′ by a RecA-enhanced proteolytic mechanism.

The greatest divergence between the two bacterial *umuDC* sequences obtained in the region encoding the 24-amino-acid N-terminal portion of UmuD. The corresponding region in MucA was also highly divergent in comparison with either of the two UmuD versions. There appears to be considerable latitude in the primary structure of this region, as if there were few constraints in the design of the N-terminal portion of the substrate for RecA-mediated autoproteolytic cleavage (15, 24).

Why is *S. typhimurium* less mutable than *E. coli*? The sequencing and mapping data show that *S. typhimurium umuDC* was not disrupted by the 10-min chromosomal inversion (a speculative suggestion offered by Walker et al. [63] as a possible explanation for the poor mutability of *S. typhimurium*). Expression of this operon might, however, have been affected by its new location, which is 10 min farther from *oriC* and probably inverted with respect to operon transcription. Chromosome position per se is not believed to significantly influence gene expression. For example, there is no evidence that the inversion event in the 25- to 35-min region affected *trp* operon function (8). Furthermore, small shifts in map position or changes in transcription orientation did not have a large effect on gene expression (47).

The cloned *umuDC* operon from *S. typhimurium* restored considerable levels of UV-induced mutagenesis to *E. coli umuC* and *umuD* mutants but less than that observed when *umuDC* from *E. coli* was introduced. The *S. typhimurium*

umuDC clone also failed to restore as much UV resistance to *E. coli umuD* or *umuC* mutants as did the *E. coli umuDC* clone. Although the uncertainty of copy number, level of expression, and efficiency of UmuD activation in these experiments confounds a quantitative interpretation of the results, the data are consistent with the physiological observations that *S. typhimurium* is less efficient in SOS mutagenesis and repair than *E. coli*. Direct measurements of chromosomal *umuDC* expression on the one hand and the efficiency of UmuD activation via cleavage on the other hand are required for a better understanding of the contribution of *S. typhimurium umuDC* to mutability.

One should not dismiss the possibility that the poor mutability of *S. typhimurium* is not directly related to *umuDC*. Gene products in addition to UmuD and UmuC are required for SOS mutagenesis and may interact with the *umuDC* products (11, 13). Variations in these components and their interactions with UmuD and UmuC could therefore be responsible for the mutability phenotype of *S. typhimurium*. Indirect support for this point of view is provided by the observation that in contrast to *E. coli*, *S. typhimurium* exhibits no inducible reactivation of phage treated with oxidative DNA-damaging agents (10). The SOS response is not believed to be a component of the bacterial response to oxidative damage (7, 10). Thus, *S. typhimurium* behaves as if there is a "defect" in a component that is common to two different repair pathways, only one of which involves *umuDC*.

The sequence analysis presented here did not identify a clear molecular basis for the mutability differences between *E. coli* and *S. typhimurium*. Systematic exchanges and alterations of the individual structural and regulatory components of the two bacterial *umuDC* operons will make it possible to explore the significance of the sequence differences. The amino acid differences between the *umuDC* products—for example, nonconservative substitutions in the *S. typhimurium* version that lie within tracts of amino acid sequence identities between *E. coli* UmuDC and MucAB—suggest one focus for molecular genetic investigations. Site-directed mutagenesis and construction of hybrid proteins will provide important information about the structure and biological function of the *umuDC* gene products.

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