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Salmonella typhimurium Has Two Homologous but Different *umuDC* Operons: Cloning of a New *umuDC*-Like Operon (*samAB*) Present in a 60-Megadalton Cryptic Plasmid of *S. typhimurium*

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Expression of the *umuDC* operon is required for UV and most chemical mutagenesis in *Escherichia coli*. The DNA which can restore UV mutability to a *umuD44* strain and to a *umuC122*::Tn*5* strain of *E. coli* has been cloned from *Salmonella typhimurium* TA1538. DNA sequence analysis indicated that the cloned DNA potentially encoded proteins with calculated molecular weights of 15,523 and 47,726 and was an analog of the *E. coli umuDC* operon. We have termed this cloned DNA the *samAB* (for *Salmonella* mutagenesis) operon and tentatively referred to the *umuDC* operon of *S. typhimurium* LT2 (C. M. Smith, W. H. Koch, S. B. Franklin, P. L. Foster, T. A. Cebula, and E. Eisenstadt, J. Bacteriol. 172:4964–4978, 1990; S. M. Thomas, H. M. Crowne, S. C. Pidsley, and S. G. Sedgwick, J. Bacteriol. 172:4979–4987, 1990) as the $umuDC_{ST}$ operon. The *samAB* operon is 40% diverged from the $umuDC_{ST}$ operon at the nucleotide level. Among five *umuDC*-like operons so far sequenced, i.e., the *samAB*, $umuDC_{ST}$, *mucAB*, *impAB*, and *E. coli umuDC* operons, the *samAB* operon shows the highest similarity to the *impAB* operon of TP110 plasmid while the $umuDC_{ST}$ operon shows the highest similarity to the *E. coli umuDC* operon. Southern hybridization experiments indicated that (i) *S. typhimurium* LT2 and TA1538 had both the *samAB* and the $umuDC_{ST}$ operons and (ii) the *samAB* operon was located in a 60-MDa cryptic plasmid. The $umuDC_{ST}$ operon is present in the chromosome. The presence of the two homologous but different *umuDC* operons may be involved in the poor mutability of *S. typhimurium* by UV and chemical mutagens.

In *Escherichia coli*, mutagenesis by UV and most chemical mutagens requires expression of the *umuDC* operon (11, 47, 60). Both *umuD* and *umuC* mutants are virtually nonmutable with UV and a variety of chemicals (19, 52, 53). The *umuD* and *umuC* genes are organized as an operon (11, 47) and encode proteins of 15.1 and 47.7 kDa, respectively (20, 38). Operons analogous to *umuDC* have been found in many conjugative plasmids (33, 40, 54). Two such operons, *mucAB* and *impAB*, have been cloned from plasmids pKM101 and TP110, respectively, and have been shown to encode proteins with molecular weights similar to those of the *umuDC* products (14, 38).

Expression of the *umuDC*, *mucAB*, and *impAB* operons is repressed by the LexA protein and is regulated as a part of the SOS response of *E. coli* (2, 11, 23, 54, 60, 61, 63), in which an activated form of RecA mediates the cleavage of a bond between Ala-84 and Gly-85 of LexA (22). This cleavage process is not a typical proteolytic reaction but is a conditional autodigestion of LexA in which RecA acts as a positive effector to facilitate the capacity of LexA to autodigest (21, 49). UmuD, MucA, and ImpA share homology with the carboxy-terminal region of LexA and repressors of bacteriophage λ, 434, P22, and φ80 (5, 10, 38). The activated form of RecA also mediates the cleavage of a bond between Cys-24 and Gly-25 of UmuD by a mechanism similar to that of the cleavage of LexA (8, 35, 46, 64). The carboxy-terminal fragment of UmuD, UmuD′, is necessary and sufficient for the role of UmuD in UV mutagenesis (35). Recent biochemical evidence has suggested that UmuC can form a complex with a homodimer of UmuD′ (64). Although various models have been proposed, the biochemical role of the complex of UmuC and the homodimer of UmuD′ in mutagenesis in *E. coli* is not yet clarified (4, 7, 12, 16, 64).

Several reports suggest that the closely related species *Salmonella typhimurium* has an SOS regulatory system which resembles that of *E. coli* (25, 30, 36, 37, 39, 45). *S. typhimurium*, especially its derivatives containing the pKM101 plasmid, has widely been used in the Ames test for the detection of environmental mutagens and carcinogens (1, 30). It is known, however, that if the pKM101 plasmid is eliminated, *S. typhimurium* itself shows a much weaker mutagenic response to UV and some chemical mutagens than does *E. coli* (30, 45, 48, 59). In fact, certain potent mutagens such as furylfuramide and methyl methanesulfonate are either nonmutagenic or weakly mutagenic to *S. typhimurium* in the absence of pKM101, whereas they are strongly mutagenic to *S. typhimurium* in the presence of the pKM101 plasmid as well as to *E. coli* (30). Moreover, Weigle reactivation of UV-irradiated phage P22 is also poor in *S. typhimurium* (59). Two reports have suggested that *S. typhimurium* is nonmutable by UV (45, 48). The low mutability, as well as the poor Weigle reactivation, can be restored to levels comparable to those of *E. coli* by introducing the plasmid carrying the *E. coli umuDC* operon or the *mucAB* operon (26, 30, 34, 59). Incorporation of the *E. coli* chromosome containing the *umuDC* genes into *S. typhimurium* increases the UV mutability of this organism (48). These lines of evidence suggest that *S. typhimurium* is deficient in the function of *umuDC* genes.

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid[a]	Description[b]	Source
E. coli		
XL1-Blue	*endA1 hsdR17*(r_K^- m_K^+) *supE44 thi-1 recA1* λ^- *gyrA96 relA1* (*lac*)(F' *proAB*$^+$ *lacI*^{q} *lacZ* ΔM15 Tn*10*)	Stratagene
GW3200	As AB1157, but has *umuD44*	G. C. Walker
GW2100	As AB1157, but has *umuC122*::Tn*5*	G. C. Walker
S. typhimurium		
LT2	Prototrophic	K. E. Sanderson
TA1538	*hisD3052*	B. N. Ames
TA2659	*hisG428*	B. N. Ames
Plasmids		
pSE117	Derivative of pBR322 with the *umuDC* operon of *E. coli*	G. C. Walker
pGW2101	Derivative of pZ150 with the *umuDC* operon of *E. coli*	G. C. Walker
pYQ100	60-MDa cryptic plasmid in *S. typhimurium* LT2	S. Murayama
pYG8011	As pBR322, but has a 12.8-kb fragment of TA1538 DNA carrying the *samAB* operon	This study
pYG8020	As pBluescript KS+, but has a 3.2-kb *Eco*RV (9.1 kb)–*Eco*RV (12.3 kb) fragment of pYG8011 carrying the *samAB* operon	This study
pYG8021	As pYG8020, but the direction of the insert DNA is opposite	This study
pYG8020-74	As pYG8020, but its insert DNA has a deletion of about 1 kb from the *Eco*RV site (12.3 kb)	This study
pYG8020-90	As pYG8020, but its insert DNA has a deletion of about 1.9 kb from the *Eco*RV site (12.3 kb)	This study
pYG8021-1	As pYG8021, but its insert DNA has a deletion of about 1 kb from the *Eco*RV site (9.1 kb)	This study
pYG8030	As pBR322, but its *Eco*RI-*Bam*HI region is replaced by a 4-kb *Eco*RI-*Bam*HI fragment of *S. typhimurium* LT2 carrying the $umuDC_{ST}$ operon	This study

[a] TA strains are derived from *S. typhimurium* LT2 and have the genotype *gal* Δ(*chl uvrB bio*) *rfa*. Detailed information on pSE117 and pGW2101 is in references 10 and 33, respectively.

[b] Numbers in parentheses indicate the map position of each restriction site in pYG8011 (Fig. 2). Pictorial representations of pYG8020-74, pYG8020-90, and pYG8021-1 are shown in Fig. 3.

Recent genetic experiments suggested, however, that *S. typhimurium* has a gene functionally homologous to the *E. coli umuC* gene, since UV mutability of *S. typhimurium* TA2659 was increased when the plasmid carrying the *E. coli umuD*$^+$*C* gene but not that carrying the *E. coli umuDC*$^+$ gene was introduced into this strain (15). Similar results with *S. typhimurium* LT2 were reported (50). Furthermore, the DNA sequence of *S. typhimurium* LT2 which can hybridize to the *E. coli umuDC* sequence has been cloned (58) and its nucleotide sequence has been determined (51, 57). The cloned *umuDC* of *S. typhimurium* LT2 and the *E. coli umuDC* are 71% homologous at the nucleotide level. The plasmid carrying the *umuDC* operon of LT2 restored UV mutability to both *umuD* and *umuC* mutants of *E. coli* (51). Those findings raised the new question of why the ability of such a functional *umuDC* operon is partially or totally suppressed in *S. typhimurium*.

In order to clarify the relation between a *umuDC* operon and the poor mutability of *S. typhimurium*, we have independently screened the genes of *S. typhimurium* TA1538 which can restore UV mutability to a *umuC122*::Tn*5* strain of *E. coli*. Consequently, we have cloned a new *umuDC*-like operon which is 40% diverged from the aforementioned *umuDC* operon of *S. typhimurium* LT2 at the nucleotide level. Since we have cloned this DNA from *S. typhimurium* and this DNA appears to be involved in mutagenesis, we have termed it the *samAB* (for *Salmonella* mutagenesis) operon. We have tentatively referred to the *umuDC* operon cloned from *S. typhimurium* LT2 (51, 57) as the $umuDC_{ST}$ operon. On the basis of the results of the Southern hybridization experiments, we have concluded that both *S. typhimurium* LT2 and TA1538 have two sets of *umuDC* operons, i.e., the *samAB* operon in a 60-MDa cryptic plasmid and the $umuDC_{ST}$ operon in the chromosome (51). The possible implications of the existence of the two homologous *umuDC* operons in the poor mutability of *S. typhimurium* against UV and chemical mutagens are discussed.

MATERIALS AND METHODS

Bacterial strains and plasmids. All bacterial strains and plasmids used in this study are listed in Table 1.

Media and culture. LB broth and agar (27) were used for routine bacterial culture and supplemented, when necessary, with 50 μg of ampicillin per ml for plasmid selection and maintenance. The semienriched-medium agar plates used for a UV-induced reversion assay of *argE3* to Arg$^+$ of *E. coli* contained the following: M9 salts (32); 0.2% glucose; 2 mM $MgCl_2$; 0.1 mM $CaCl_2$; 40 μg each of threonine, leucine, isoleucine, proline, valine, and histidine per ml; 5 μg of thiamine per ml; 1 μg of arginine per ml; and 1.5% Bacto-agar. Vogel-Bonner minimal agar plates and top agar used for the reversion assay of *hisG428* to His$^+$ of *S. typhimurium* TA2659 were prepared as previously described (29).

Isolation of genomic DNA and plasmid DNA. To avoid semantic confusion, we have referred to total cellular DNA, including the 60-MDa cryptic plasmid, as genomic DNA in this study. The genomic DNA of *S. typhimurium* LT2 and TA1538 was prepared by the method of Marmur (28). The 60-MDa cryptic plasmid of *S. typhimurium* LT2 was isolated by the method of Kado and Liu (18) and was designated pYQ100. A method to construct a restriction map of pYQ100 will be published elsewhere. Other multicopy plasmids were isolated by the method of Birnboim and Doly (6).

Cloning of the *samAB* operon. A gene library of *S. typhimurium* TA1538 was constructed by ligating partially *Sau*3AI digested genomic DNA, whose size is about 10 kilobase pairs (kb), with *Bam*HI-digested pBR322 (62). The

library DNA was modified by introducing it into an XL1-Blue strain (r_K^- m_K^+) of *E. coli*. More than 30,000 transformants of XL1-Blue were collected, and plasmid DNA was extracted. An AB1157 *umuC122*::Tn*5* strain (GW2100) of *E. coli* was transformed with the modified library DNA, and ampicillin-resistant colonies were selected. Each transformant was picked up and patched onto semienriched-medium agar plates to test its ability to induce reversion of *argE3* to Arg^+ after UV irradiation (a patch mutagenesis assay). The plates were irradiated with UV at 20 J/m^2 and incubated for 2 days at 37°C. Plasmid DNA was reisolated from the master colony of apparent UV-mutable transformants and was introduced into a fresh *umuC122*::Tn*5* background. The plasmid DNA which restored UV mutability to a *umuC122*::Tn*5* strain was selected by checking the second transformants for their UV mutability by the patch mutagenesis assay. The selected plasmid (pYG8011) was introduced into both a *umuD44* strain of *E. coli* (GW3200) and a TA2659 strain of *S. typhimurium*. The resulting transformants as well as a *umuC122*::Tn*5* strain containing pYG8011 were subjected to a quantitative UV-mutagenesis assay.

Quantitative UV-mutagenesis assay. Log-phase cells (A_{600}, 0.5 to 0.7) were washed three times with cold saline (0.85% sodium chloride solution) and resuspended. A portion (0.2 ml) of the UV-irradiated suspension was spread on semienriched-medium agar plates with a sterile glass rod (*E. coli*) or spread on the Vogel-Bonner minimal agar plates with 2 ml of molten top agar (*S. typhimurium*). For counting the surviving cells, the cell suspension was diluted 10^5-fold with cold saline and 0.1 ml of the diluted suspension was spread on semienriched-medium agar plates or on the Vogel-Bonner agar plates with the top agar. Induced-mutation frequency was calculated by dividing the number of induced revertants per plate by that of surviving cells per 0.2 ml of the undiluted suspension. All plates were incubated at 37°C for 2 days, except the plates spread with *E. coli* cells containing pBluescript vector or its derivatives were incubated at 30°C for 3 days.

Labeling of plasmid-coded proteins in maxicells. The maxicell method of Sancar et al. (41) was used to label the proteins encoded by plasmids pGW2101 and pYG8020 and its derivatives in the CSR603 strain with [^{35}S]methionine. Samples were run on a 12% sodium dodecyl sulfate (SDS)-polyacrylamide gel and visualized by fluorography.

DNA sequencing. Sets of deletion derivatives of pYG8020 and pYG8021 were constructed by using the exonuclease III-mung bean nuclease digestion protocol from Stratagene. Double-stranded sequencing of both strands of the 1.9-kb region containing the *samAB* operon was carried out by the dideoxy-chain termination technique of Sanger et al. (44) with the Sequenase Sequencing Kit version II (U.S. Biochemical Co., Cleveland, Ohio). Analysis of sequencing data was carried out with SDC-Genetyx software (SDC Software Development Co., Tokyo, Japan).

Southern blot analysis. Genomic DNA and plasmid DNA were digested with various restriction enzymes shown in the legends of Fig. 7 and 8, and digested DNA samples were run on a 0.7% agarose gel. DNA in the gel was denatured in situ and transferred to a nylon membrane (Hybond-N, Amersham-Japan, Tokyo, Japan). The filter was irradiated with UV and then incubated overnight at 65°C in a solution containing 5× SSC (1× SSC is 150 mM sodium chloride and 15 mM sodium citrate [pH 7.0]), 1 mM EDTA, 1% SDS, and 10 μg of yeast RNA per ml. The filter was then hybridized to ^{32}P-labeled probe DNA overnight at 65°C in the solution described above. Following the hybridization, the filter was washed twice for 15 min at 60°C in 0.5× SSCP (1× SSCP is 150 mM sodium chloride, 15 mM sodium citrate, 20 mM sodium phosphate monobasic [pH 6.5]) and then autoradiographed.

DNA probes for hybridization. The following DNA fragments were labeled with [^{32}P]dCTP (DuPont, NEN Research Products, Boston, Mass.) by using the Random Primer DNA Labeling Kit (Takara Shuzo Co., Kyoto, Japan).

(i) DNA fragment containing the *samAB* operon. The purified DNA of pYG8011 (6.0 μg) was digested with *Eco*RV followed by 1% agarose gel electrophoresis. The 3.2-kb DNA band was excised from the gel, and the DNA was purified by using the Geneclean II Kit (Bio 101, La Jolla, Calif.).

(ii) DNA fragment containing the *umuDC*$_{ST}$ operon. The *umuDC*$_{ST}$ operon has been cloned from *S. typhimurium* LT2 according to the method of Thomas and Sedgwick (58). The purified DNA of pYG8030 (20 μg) containing a 4-kb *Eco*RI-*Bam*HI fragment carrying the *umuDC*$_{ST}$ operon was digested with both *Hin*dIII and *Sal*I and then underwent 1% agarose gel electrophoresis. The 2.2-kb band was excised and purified as described above.

Nucleotide sequence accession number. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank Nucleotide Sequence data bases under the accession number of D90202.

RESULTS

Cloning of the genes of *S. typhimurium* TA1538 which can restore UV mutability to a *umuC* mutant of *E. coli* (cloning of the *samAB* operon). Of about 1,000 ampicillin-resistant transformants of an AB1157 *umuC122*::Tn*5* strain (GW2100) with the library DNA of TA1538, we selected two candidates which apparently showed UV mutability in the patch mutagenesis assay. To confirm that the UV-mutable phenotype is due to the presence of the plasmids carrying a part of genomic DNA of TA1538, we have extracted the plasmids from the candidates and reintroduced them into a fresh *umuC122*::Tn*5* background. Although one plasmid did not reproduce the suppression of UV nonmutability, the other plasmid, which we designated pYG8011, did restore the UV mutability to a *umuC122*::Tn*5* strain in the patch mutagenesis assay.

In order to validate the results of the initial screening assay and to compare the ability of pYG8011 with that of pSE117, which carries the *E. coli umuDC* operon, we have carried out the quantitative UV-mutagenesis assays using an AB1157 *umuD44* strain, an AB1157 *umuC122*::Tn*5* strain, and an *S. typhimurium* TA2659 strain containing the pYG8011, pSE117, or pBR322 plasmid. Introduction of pYG8011 restored UV mutability to both a *umuD44* strain and a *umuC122*::Tn*5* strain to almost the same extent, but the levels of UV mutagenesis were about one-half or one-third of those observed for the same strains containing pSE117 (Fig. 1A and B). Similar results were obtained when *hisG4* instead of *argE3* was used as a marker of reverse mutation (data not shown). Neither pYG8011 nor pSE117 appeared to enhance the survival of UV-irradiated host strains. They did not increase the spontaneous mutation frequency. The control strains, a *umuD44* strain containing pBR322 and a *umuC122*::Tn*5* strain containing pBR322, were nonmutable even at a UV dose of 20 J/m^2.

In contrast to that of the *E. coli* strains, the UV mutability of a *S. typhimurium* TA2659 strain containing pYG8011 was about 80% that of a TA2659 strain containing pSE117 (Fig.

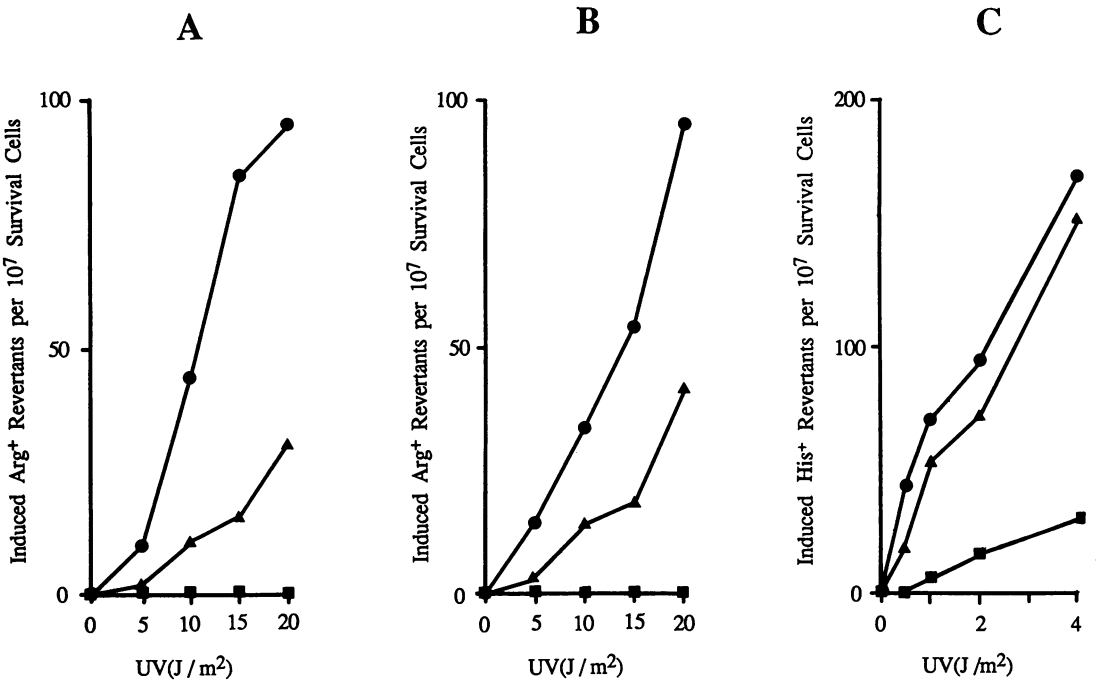

FIG. 1. Effect of pYG8011 on the frequency of Arg^+ reversion induced by UV irradiation in an AB1157 *umuD44* strain (A), an AB1157 *umuC122*::Tn*5* (GW2100) strain (B) and a *S. typhimurium* TA2659 strain (C). ▲, pYG8011; ●, pSE117; ■, pBR322.

1C). The control strain, TA2659 containing pBR322, showed about a fivefold-lower UV mutability than did TA2659 containing pYG8011. The number of His^+ revertants of TA2659 containing pYG8011 (216 His^+ revertants per plate at a UV dose of 2 J/m^2) was slightly higher than that of TA2659 containing pSE117 (157 His^+ revertants per plate at the same UV dose) when an overnight culture washed with saline instead of a log-phase culture was used.

Determination of the minimum essential region of cloned DNA for restoration of UV mutability. A restriction map of pYG8011 was constructed by digesting the plasmid with several restriction enzymes (Fig. 2). The plasmid (17.2 kb) was composed of pBR322 (4.4 kb) and genomic DNA of TA1538 (12.8 kb). To determine the region necessary for the suppression of the UV-nonmutable phenotypes of *umuDC* mutants of *E. coli*, we have partially digested pYG8011 with *Eco*RV and constructed a set of deletion derivatives. Upon checking the UV mutability of such deletion plasmids, we have suggested that the 3.2-kb region spanning from the map position of 9.1 kb (the third *Eco*RV site) to that of 12.3 kb (the fourth *Eco*RV site) is necessary for the restoration of UV mutability of a *umuC122*::Tn*5* strain. The restriction map within the 3.2-kb region was different from those of the *E. coli umuDC*, *mucAB*, and *impAB* operons (20, 38, 54) and of the $umuDC_{ST}$ operon (58).

To further define the minimum DNA region necessary for the restoration of UV mutability, we have subcloned the 3.2-kb DNA fragment into an *Eco*RV site of pBluescript KS+ vector. The resulting plasmids, pYG8020 and pYG8021, had the same insert DNA with opposite orientations. The two plasmids efficiently suppressed the UV nonmutability of both a *umuD44* strain and a *umuC122*::Tn*5* strain (data not shown). More than 100 deletion derivatives were prepared from pYG8020 and pYG8021 according to the exonuclease III-mung bean nuclease digestion protocol. Among these deletion plasmids, 27 and 21 subcloned plasmids derived from pYG8020 and pYG8021, respectively, were introduced into a *umuD44* strain and a *umuC122*::Tn*5* strain and were checked by the patch mutagenesis assay for their ability to complement the mutations (Fig. 3).

In the case of derivatives of pYG8020, the six longest subclones could complement both a *umuD44* strain and a *umuC122*::Tn*5* strain, while the plasmids which had deletions of more than 600 bp from the *Eco*RV site on the right in Fig. 3 no longer complemented both *umu* mutations. On the other hand, the six longest subclones of pYG8021 complemented both mutations, whereas the plasmids whose insert DNA was digested more than 700 bp from the *Eco*RV site on the left in Fig. 3 no longer complemented both mutations. The *Eco*RV sites shown on the right and left in Fig. 3 correspond to the map positions of 12.3 and 9.1 kb, respectively, of pYG8011 (Fig. 2). Thus, we suggested that the 1.9-kb region shown as an open box in Fig. 3 is the minimum essential region for the suppression of the nonmutable phenotype of both *umuD44* and *umuC122*::Tn*5* strains of *E. coli*.

Identification of gene products (SamA and SamB) involved in suppression of UV nonmutability of *umu* mutants of *E. coli*. To identify the gene products involved in the suppression of UV nonmutability of *E. coli umu* mutants, proteins synthesized in maxicells containing pYG8020 or pGW2101 were labeled with [^{35}S]methionine and subjected to SDS-polyacrylamide gel electrophoresis followed by fluorography. Plasmid pYG8020 produced two proteins with approximate molecular masses of 17 and 46 kDa, respectively, as did pGW2101, which carries the *E. coli umuDC* operon (Fig. 4A). The efficiency of expression of pYG8020 was comparable to that of expression of pGW2101. Plasmid pYG8021, having the same insert DNA as pYG8020 but with the opposite orientation, produced the proteins with same size as those of pYG8020 (data not shown). From these results together with those shown in Fig. 1, we suggested that the

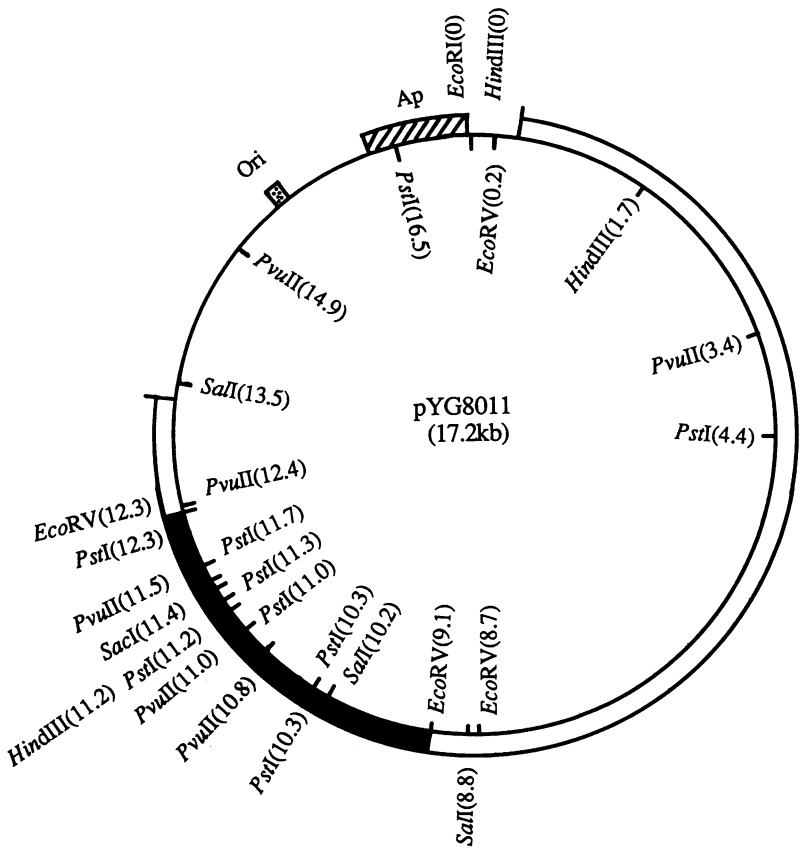

FIG. 2. Restriction map of pYG8011. The *Eco*RI restriction site derived from pBR322 was assigned the map position of 0 kb of the 17.2-kb pYG8011 map. A DNA fragment (12.8 kb) derived from *S. typhimurium* TA1538 was inserted into the *Bam*HI site of pBR322. The *samAB* genes were located in a 3.2-kb *Eco*RV-*Eco*RV fragment (■), which was used for subcloning into pBluescript KS+. Ori, DNA replication origin; Ap, ampicillin resistance gene.

DNA we have cloned from *S. typhimurium* TA1538 contains the two genes which are analogous to the *E. coli umuD* and *umuC* genes. Since we have cloned this DNA from *S. typhimurium* and this DNA appears to be involved in mutagenesis, we have termed the cloned genes *samAB*.

To address the question of whether the *samAB* genes are organized as an operon, we have examined the proteins encoded by subclones derived from pYG8020 and pYG8021 using the maxicell technique. Plasmid pYG8020-74, whose insert DNA was deleted about 1 kb from the *Eco*RV site on the right in Fig. 3, produced the smaller protein (SamA) but no longer produced the intact bigger protein (SamB) (Fig. 4B). Instead, it produced a protein with an approximate molecular mass of 32 kDa. Plasmid pYG8020-90, whose insert DNA was deleted about 1.9 kb from the right *Eco*RV site, produced neither the SamA nor the SamB protein. These results suggested that the *samA* gene and the *samB* gene were located on the left side and right side, respectively, of the 1.9-kb region. The fact that pYG8021-1, whose insert DNA was deleted about 1 kb from the *Eco*RV site on the left in Fig. 3, did not produce the two proteins suggests that the *samAB* genes are also organized as an operon where the SamA protein is expressed first and that its promoter is probably located in the leftmost region of the 1.9-kb essential sequence.

DNA sequence of the *samAB* operon. We have determined the nucleotide sequence of both strands of the 1.9-kb region which carries the *samAB* operon (Fig. 5). The nucleotide sequence of the 1.9-kb region contains two continuous reading frames of 420 and 1,272 bp. The open reading frames corresponded to the positions of the *samA* and *samB* genes, respectively (as deduced from the maxicell experiments), and potentially encoded proteins of 140 and 424 amino acids with calculated molecular weights of 15,523 and 47,726, respectively. A potential SOS box sequence for the binding of LexA and a potential ribosome-binding site have been found upstream of the *samA* gene. In *E. coli*, there is a second but apparently nonfunctional SOS box around the −35 region (20, 38, 60). Interestingly, such a second SOS box was also present upstream of the *samA* gene from nucleotides 39 to 56. Like the *E. coli umuDC* operon, the *samA* and *samB* genes overlap by 1 bp. The *samA* gene encodes the sequence of Ala-24 and Gly-25 at a putative cleavage site by an activated form of RecA, as do the *mucAB* and *impAB* operons (5, 24, 38). In addition, it encodes Ser-61 and Lys-98, which are highly conserved not only in UmuD, MucA, and ImpA but also in LexA and the phage repressors and are potential catalytic sites for their autodigestion (5, 10, 35, 38, 49). No special codon usage was found in the *samAB* operon.

Similarity of *samAB* to other related operons. Thomas et al. (57, 58) and Smith et al. (50, 51) have independently cloned and sequenced another analog of *umuDC* genes from *S. typhimurium* LT2. It is of interest, therefore, to compare the

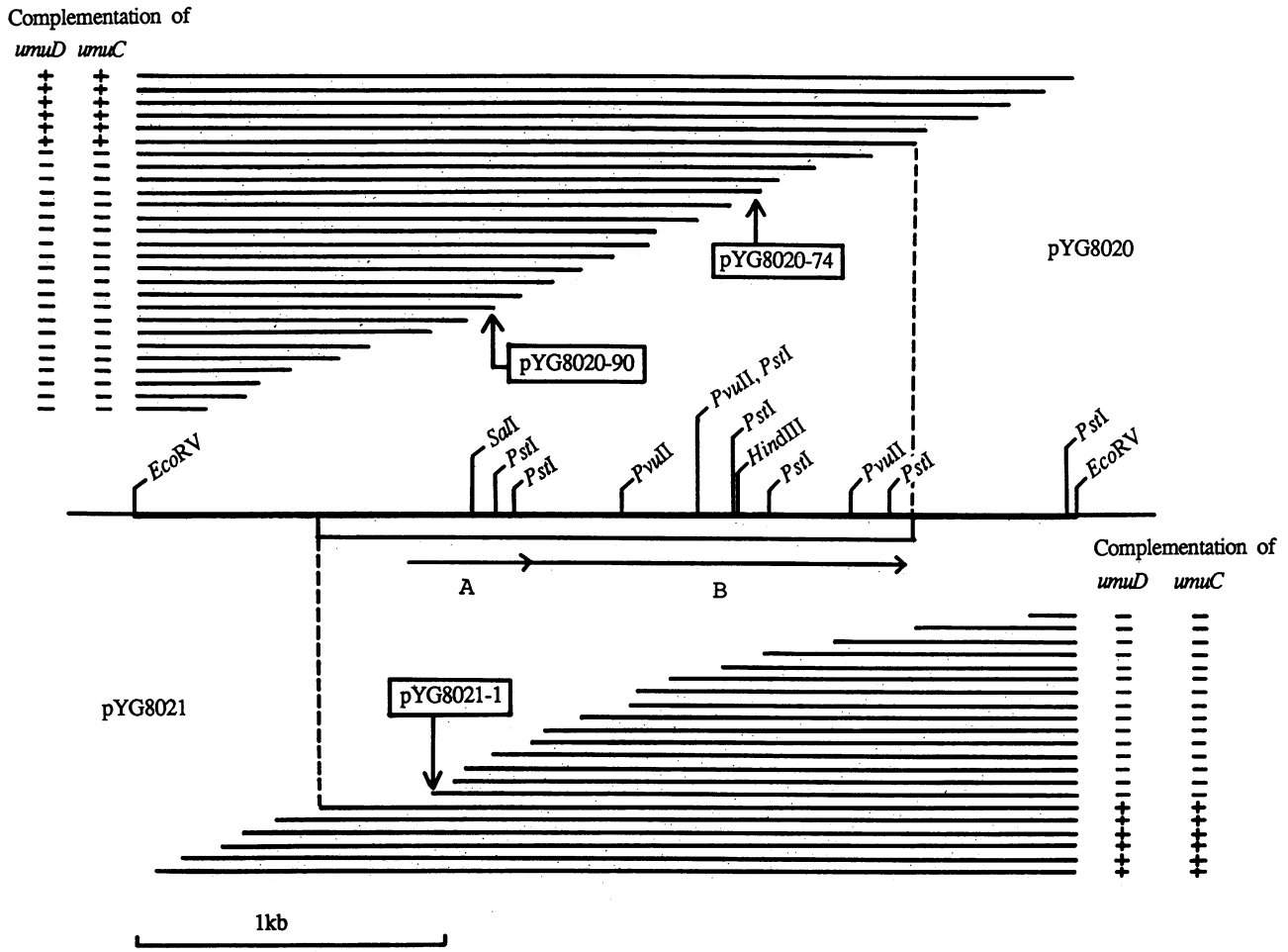

FIG. 3. Partial restriction map of the 3.2-kb *Eco*RV-*Eco*RV region of pYG8020 and of pYG8021 and predicted locations of the *samAB* genes. Both pYG8020 and pYG8021 are plasmids derived from pBluescript KS+ (Stratagene, La Jolla, Calif.) and have the same insert DNA (the 3.2-kb *Eco*RV-*Eco*RV fragment of pYG8011) in opposite orientations. Bars represent DNA of the 3.2-kb region remaining after exonuclease III-mung bean nuclease digestion. + and − indicate the ability or inability of each subclone to complement an AB1157 *umuD44* strain or an AB1157 *umuC122*::Tn*5* strain. The complementation assay was carried out by the patch mutagenesis assay as described in Materials and Methods. The plasmids containing deleted insert DNA, pYG8020-74, pYG8020-90, and pYG8021-1, were used for the ^{35}S-labeling experiments shown in Fig. 4.

nucleotide and amino acid sequences of the *samAB* operon with those of the *umuDC* operon cloned from LT2 as well as those of the *mucAB*, *impAB*, and *E. coli umuDC* operons (20, 24, 38). We have tentatively referred to the *umuDC* operon of *S. typhimurium* LT2 (51, 57) as the $umuDC_{ST}$ operon.

The *samAB* and the $umuDC_{ST}$ operons were 60% homologous at the nucleotide level (Fig. 6A and B). The predicted amino acid sequences of the UmuD-like proteins encoded by the two operons were 49% homologous; those of the UmuC-like proteins encoded by them were 63% homologous (Fig. 6C to F). Interestingly, the *samAB* operon showed more similarity to the *impAB* operon of TP110 plasmid than to the other three related operons (Fig. 6A), whereas the $umuDC_{ST}$ operon showed the most similarity to the *E. coli umuDC* operon (Fig. 6B). Similar relationships were observed at the amino acid level: the SamA and SamB proteins showed the most similarity to the ImpA and ImpB proteins, respectively (Fig. 6C and D), while the UmuD and UmuC proteins encoded by the $umuDC_{ST}$ operon showed the most similarity to the *E. coli* UmuD and UmuC proteins, respectively (Fig. 6E and F). Both the *samAB* and $umuDC_{ST}$ operons showed the least similarity to the *mucAB* operon (Fig. 6A and B). These results indicated that the two *umuDC* operons from *S. typhimurium* are homologous but different.

***S. typhimurium* has two sets of *umuDC* operons.** Since the *samAB* and $umuDC_{ST}$ operons have been cloned from different strains of *S. typhimurium*, there was a possibility that *samAB* operon was specific to the TA1538 strain and the $umuDC_{ST}$ operon was specific to the LT2 strain. To address the question of whether the *samAB* operon is also present in an LT2 strain, multiple restriction enzyme digests of genomic DNA extracted from LT2 and TA1538 strains were subjected to Southern hybridization using a probe containing the *samAB* sequence (Fig. 7A and B). Although hybridization to the control track of pYG8011 was strongest, hybridization to the filters containing the digests of LT2 and TA1538 gave discrete bands. Relatively intense bands at 3.2 kb were observed in *Eco*RV digests of DNA of both LT2 and TA1538 along with the control track of *Eco*RV digests of pYG8011 DNA. Characteristic bands at the molecular sizes of 400 to 700 bp were observed in *Pst*I digests of DNA of

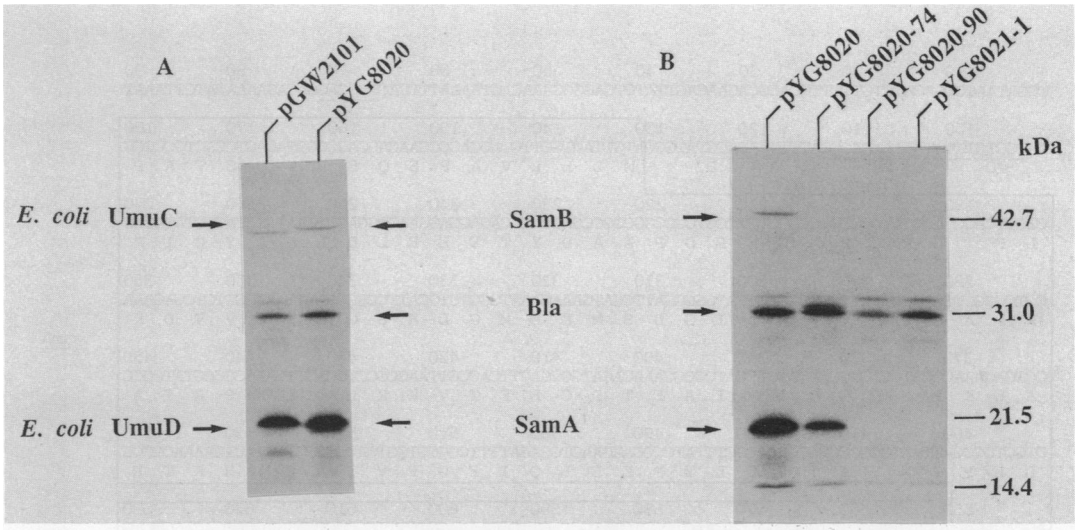

FIG. 4. [^{35}S]methionine-labeled proteins synthesized in maxicells containing pGW2101, pYG8020, and derivatives of pYG8020 and of pYG8021. (A) pGW2101 containing the *umuDC* operon of *E. coli* and pYG8020 containing the *samAB* operon; (B) pYG8020 (*samA*$^+$*B*$^+$), pYG8020-74 (*samA*$^+$*B*), pYG8020-90 (*samAB*), and pYG8021-1 (*samAB*). Pictorial representations of the deleted insert DNA of pYG8020-74, pYG8020-90, and pYG8021-1 are shown in Fig. 3.

both LT2 and TA1538 along with the control track of *Pst*I digests of pYG8011. These results indicated that (i) the *samAB* operon is not an artificial product of DNA rearrangement which occurred during manipulation and (ii) the *samAB* operon is present not only in a TA1538 strain but also in an LT2 strain.

To address the question of whether the $umuDC_{ST}$ operon cloned from LT2 is also present in a TA1538 strain, we have employed the 2.2-kb *Hin*dIII-*Sal*I fragment of the $umuDC_{ST}$ operon as a probe for hybridization to the identical filters used in the above experiments (Fig. 7C and D). The probe DNA hybridized to both LT2 DNA and TA1538 DNA. The electrophoretic mobilities of the positive bands were consistent with those of the hybridization bands reported by Thomas and Sedgwick (58). In contrast to the probe DNA carrying the *samAB* sequence, the probe DNA having the $umuDC_{ST}$ sequence did not hybridize to the plasmid DNA of pYG8011. These results indicated that both *S. typhimurium* LT2 and TA1538 have two sets of *umuDC* operons, i.e., the *samAB* operon and the $umuDC_{ST}$ operon.

The *samAB* operon is present in the 60-MDa cryptic plasmid of *S. typhimurium*. The original line of *S. typhimurium* LT2 contained a specific plasmid which has been called the cryptic plasmid, the virulence plasmid, the 100-kb plasmid, the 60-MDa plasmid, pSLT, or pYQ100 (43). On the other hand, many bacterial plasmids encode analogs of the *E. coli* *umuDC* operon and thus have the effect of increasing the UV mutability of host strains (54). It is of interest, therefore, to see whether one of the two sets of *umuDC* operons of *S. typhimurium* is located in the cryptic plasmid. To address this question, the cryptic plasmid, pYQ100, was digested with several restriction enzymes and subjected to agarose gel electrophoresis followed by transfer of the DNA to a nylon membrane filter. Two DNA probes carrying the *samAB* sequence or the $umuDC_{ST}$ sequence were examined for their ability to hybridize to the DNA bound on the membrane filter. The probe DNA carrying the *samAB* sequence strongly hybridized to the plasmid DNA bound on the filter (Fig. 8A). The electrophoretic mobilities of the positive bands all corresponded to those of the plasmid DNA bands visualized by ethidium bromide staining on the agarose gel, suggesting that the positive bands were not due to the contaminated chromosome DNA but were due to the plasmid DNA itself. One of us (S.M.) has already constructed a restriction map of pYQ100 using *Hin*dIII, *Sal*I, and *Eco*RI. By comparing the apparent molecular weights of the positive bands in Fig. 8A with those of the restriction fragments of pYQ100, we have assigned the *samAB* operon to a region around the junction between the H4 and H8 fragments of the cryptic 60-MDa plasmid (Fig. 9). In fact, the restriction map around the H4 and H8 fragments of the cryptic plasmid was very similar to that of the *samAB* operon and its flanking region deduced from restriction enzyme analysis (Fig. 2) and Southern hybridization analysis (Fig. 7A and B).

The probe DNA carrying the $umuDC_{ST}$ sequence very weakly hybridized to the filter containing pYQ100 DNA (Fig. 8B). We have suggested that these weak bands were not due to the plasmid DNA but were due to a trace amount of chromosome DNA contaminating the preparation, because the intensity of the bands was very weak and none of the bands corresponded to the bands of plasmid DNA visualized by ethidium bromide staining on the agarose gel. It is reported that the $umuDC_{ST}$ operon is located in a region between 35.9 and 40 min on the *S. typhimurium* chromosome (51).

DISCUSSION

We have cloned the genes of *S. typhimurium* TA1538 which can restore UV mutability to a *umuD44* strain and to a *umuC122*::Tn*5* strain of *E. coli* (Fig. 1). We have identified the gene products encoded by the 1.9-kb DNA region responsible for the restoration (Fig. 3) using the maxicell technique and suggested that the genes are organized as an operon (Fig. 4). DNA sequencing analysis indicated that the 1.9-kb DNA contained two open reading frames which potentially encode proteins of 15.5 and 47.7 kDa (Fig. 5). These values were consistent with those of the products estimated by the maxicell experiments (Fig. 4). A possible

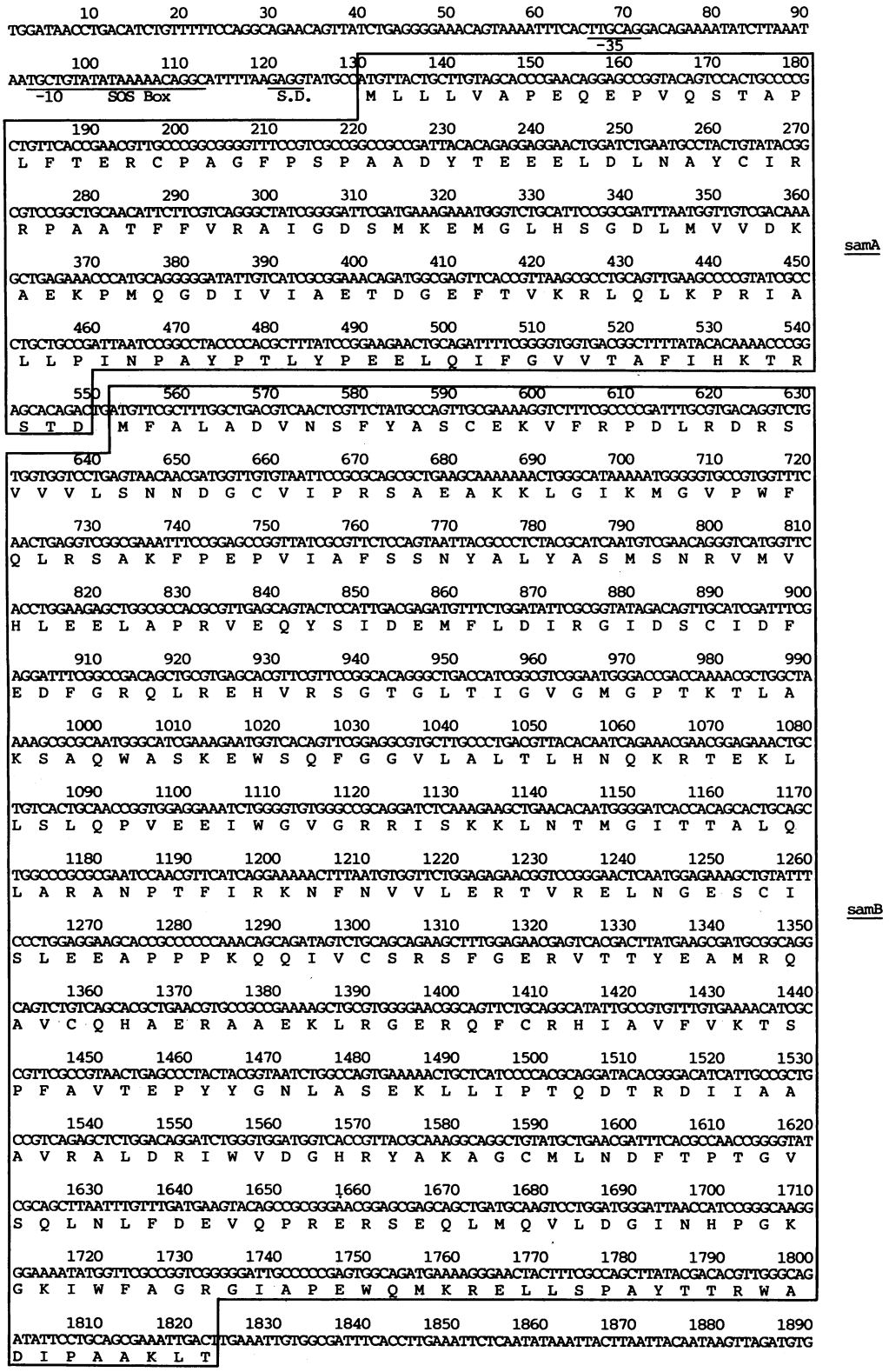

FIG. 5. Nucleotide and predicted amino acid sequences of the *samAB* operon. Two open reading frames corresponding to the *samA* and *samB* genes extend from nucleotides +130 to +549 and from nucleotides +552 to +1823, respectively. The putative SOS box, ribosome-binding site (Shine-Dalgarno [S.D.] sequence), and −10 and −35 regions are underlined.

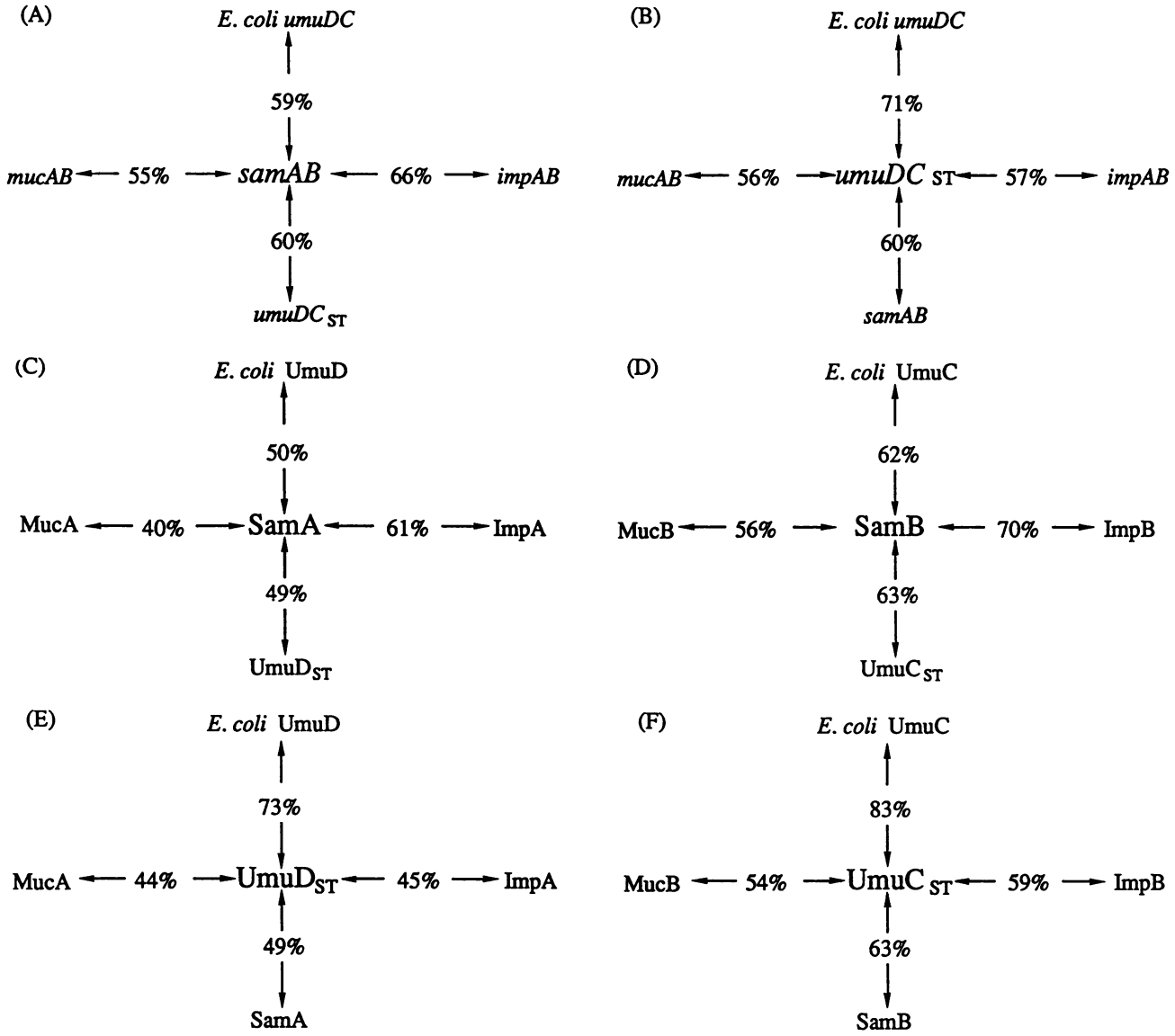

FIG. 6. Similarities among the *samAB* and *umuDC*$_{ST}$ operons and their related operons. Similarities at the nucleotide level (A and B) and at the amino acid level (C to F) are indicated as percentages.

LexA-binding site, the SOS box, has been identified in the promoter region (Fig. 5). Thus, we conclude that the cloned genes are analogs of the *E. coli umuDC* genes. We have termed the cloned genes the *samAB* and tentatively referred to the *umuDC* operon cloned from *S. typhimurium* LT2 (50, 51, 57, 58) as the *umuDC*$_{ST}$ operon.

The two *umuDC* operons of *S. typhimurium* were 40% diverged at the nucleotide level, suggesting that the two operons are homologous but different (Fig. 6). Southern hybridization experiments using each of the two *Salmonella umuDC* operons as probes demonstrated that *S. typhimurium* LT2 and TA1538 possess both of the operons (Fig. 7). The *samAB* operon is located in the 60-MDa cryptic plasmid (Fig. 8), while the *umuDC*$_{ST}$ operon is in chromosomal DNA (51). The cryptic plasmid is carried by almost all of the lines of LT2, even though the strain has been in cultures for many years and has been subjected to innumerable single-colony isolations (43). Thus, we suggest that not only TA1538 but also other derivatives of LT2, including the Ames tester strains, have the *samAB* operon along with the *umuDC*$_{ST}$ operon.

The method we have employed for screening a *umuDC* operon of *S. typhimurium* was a direct functional assay, i.e., directly searching the genes which can suppress UV nonmutability of a *umuC122*::Tn*5* strain of *E. coli*. On the other hand, the screening method which Thomas et al. (57, 58) and Smith et al. (50, 51) have employed was to look for the DNA which can hybridize to the probe DNA carrying the *E. coli umuDC* sequence followed by a functional complementation assay using the *umuC* mutant of *E. coli*. The *umuDC*$_{ST}$ and *E. coli umuDC* operons are 71% homologous at the nucleotide level, whereas the *samAB* and *E. coli umuDC* operons are 59% homologous (Fig. 6A and B). Thus, it is reasonable that Thomas et al. (57, 58) and Smith et al. (50, 51), who have employed the hybridization as a first screening method, have cloned the *umuDC*$_{ST}$ operon instead of the *samAB* operon.

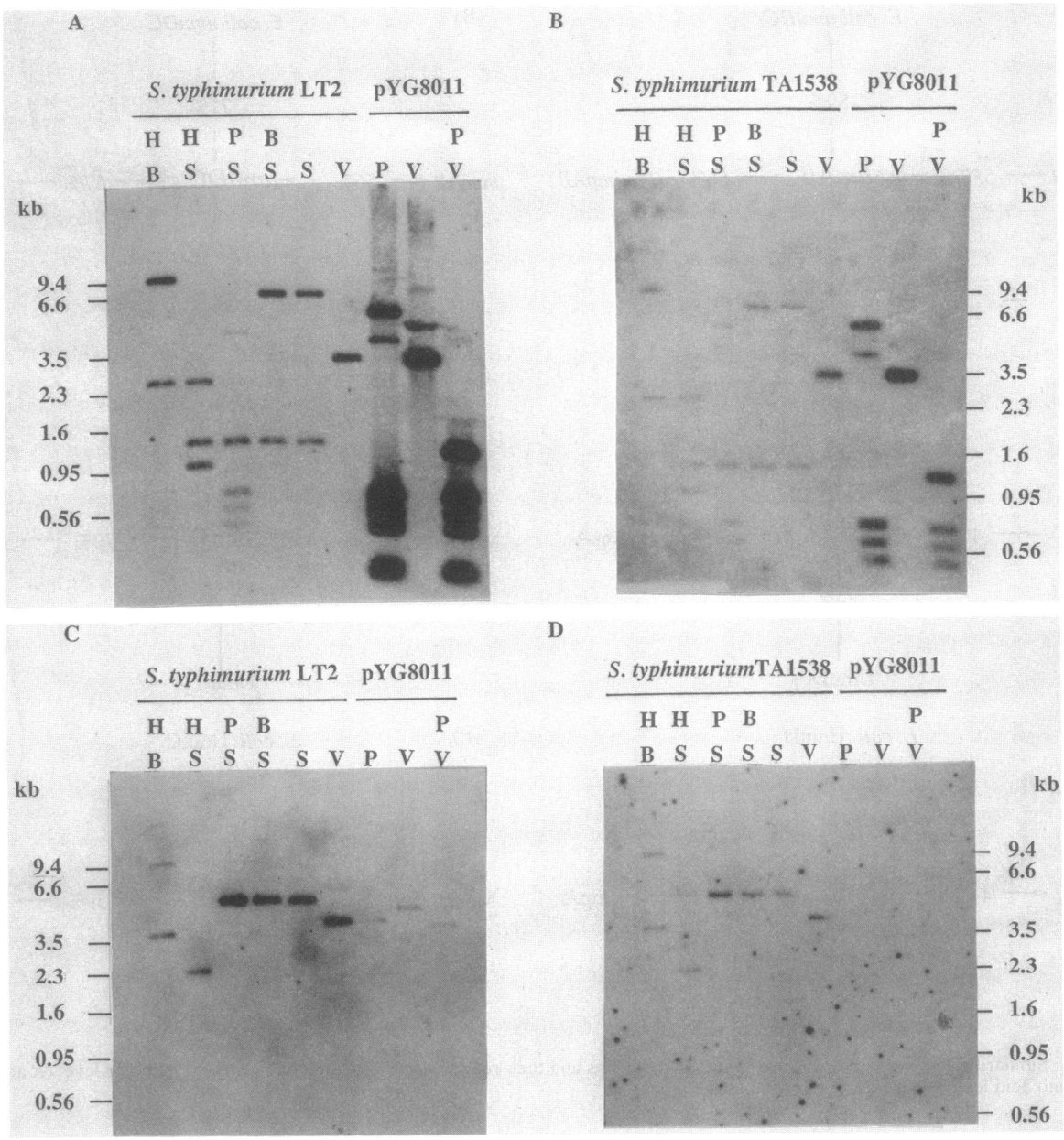

FIG. 7. Southern hybridization analysis of DNA of *S. typhimurium* LT2 and TA1538 with the probe carrying the *samAB* (A and B) or the $umuDC_{ST}$ (C and D) operon. Restriction enzymes: H, *Hin*dIII; B, *Bam*HI; S, *Sal*I; P, *Pst*I; V, *Eco*RV.

The *samAB* operon was located in the junction region between the H4 and H8 fragments of the 60-MDa cryptic plasmid of *S. typhimurium* (Fig. 9). The 20-kb region of the cryptic plasmid around the *samAB* operon is designated *repA*, a major replicon of the cryptic plasmid (31). The *repA* region also encodes incompatibility (*inc*) and partitioning (*par*) functions (31). It might be of interest to see whether the *samAB* operon plays some role in the functions of the *repA* region.

To date, five mutation-enhancing operons have been cloned either from chromosomes or from plasmids. They are the *E. coli umuDC* (20, 38), the *mucAB* (38), the *impAB* (14, 24, 54), the $umuDC_{ST}$ (51, 57), and the *samAB* operons. Each of these operons has probably evolved from a common ancestor, since the sums of the sizes of the proteins coded by the operons are very similar and the proteins show a remarkable degree of homology (Fig. 6). In particular, some operons, such as the *E. coli umuDC* and the $umuDC_{ST}$ operons, are much more similar to each other than they are to others (Fig. 6). Both the *E. coli umuDC* operon and the $umuDC_{ST}$ operon are located on chromosomal DNA: the *E. coli umuDC* operon is located at 26 min, and the $umuDC_{ST}$ operon is located in a region between 35.9 and 40 min, which corresponds to one end of a chromosome inversion of *S. typhimurium* (19, 51). It is suggested that the two *umuDC* operons were present in a common ancestor before *E. coli* and *S. typhimurium* diverged (51). In this connection, the result that the *samAB* operon shows the highest similarity to the *impAB* operon of the TP110 plasmid is intriguing (Fig. 6). This result raises the possibility that these two operons are

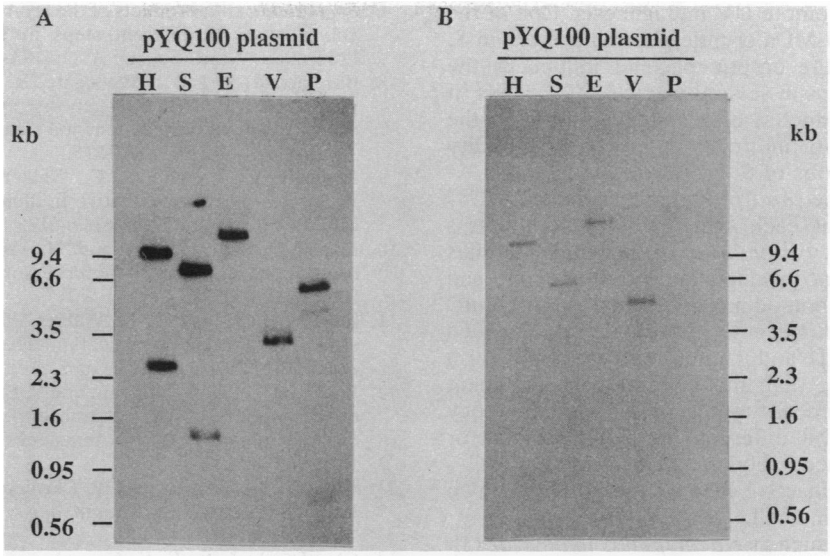

FIG. 8. Southern hybridization analysis of DNA of the 60-MDa cryptic plasmid, pYQ100, with the probe carrying the *samAB* (A) or the *umuDC*$_{ST}$ (B) operon. Restriction enzymes: H, *Hin*dIII; S, *Sal*I; E, *Eco*RI; V, *Eco*RV; P, *Pst*I.

phylogenetically related and genetically more similar to each other than they are to others. The *samAB* operon does not induce cold sensitivity in a *lexA* (Def) strain of *E. coli* even when it is on the high-copy-number vector (unpublished results). Complementation analyses suggested that the SamA produced from the *samAB* operon, like the MucA from the *mucAB* operon, cannot substitute for the UmuD of *E. coli* in an *E. coli umuD44* background (Fig. 3). SamA probably requires its cognate partner, SamB, for activity. It is of interest to see whether the *impAB* operon also has such genetic characteristics. Comparison of the nucleotide sequences and the genetic characteristics of *umuDC*-analogous operons could be useful in evaluating the evolutional relationships among them.

Why is *S. typhimurium* less mutable than *E. coli*? Introduction of plasmid pYG8011 carrying the *samAB* operon restored considerable levels of UV mutability to a *umuD44* strain and a *umuC122*::Tn*5* strain (Fig. 1A and B). Almost the same extent of restoration of UV mutability was reported when the plasmid carrying the *umuDC*$_{ST}$ operon was introduced into the *umu* mutants (51). Although the levels of restored UV mutability of the *umu* mutants were one-half or one-third of those observed with pSE117 carrying the *E. coli umuDC* operon, *S. typhimurium* has both of the *umuDC* operons (Fig. 7). Thus, if the two operons worked additionally, *S. typhimurium* should show UV mutability comparable to that of *E. coli*. Furthermore, introduction of pYG8011 enhanced the UV mutability of *S. typhimurium* TA2659 to levels which were about 80% of those observed with pSE117 (Fig. 1C). When the *samAB* operon was on the high-copy-number vector pBluescript KS+, it mediated UV mutagenesis much more efficiently than did pSE117 in a *umuD44* strain and a *umuC122*::Tn*5* strain (unpublished results). The efficiency of expression of the *samAB* operon from the high-copy-number plasmid was comparable to that of expression of the *E. coli umuDC* operon (Fig. 4A). These results suggest that both of the *umuDC* operons are active per se, but their ability to promote UV mutagenesis is partially or totally suppressed by unknown mechanisms in *S. typhimurium*.

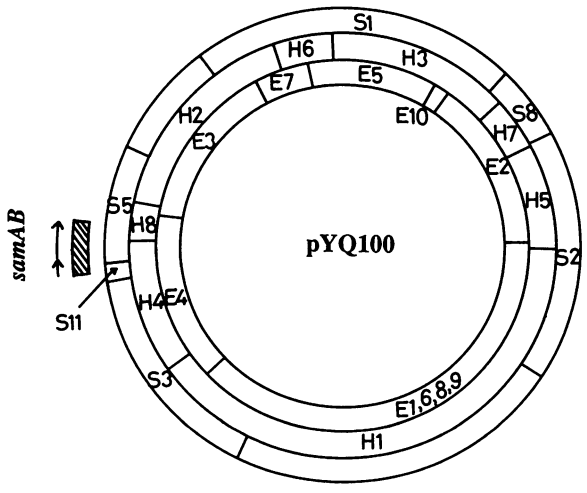

FIG. 9. Restriction map of the 60-MDa cryptic plasmid, pYQ100, and map position of the *samAB* operon. Restriction enzymes: H, *Hin*dIII; S, *Sal*I; E, *Eco*RI.

Following are possible mechanisms involved in the suppression of the ability of the two *umuDC* operons in *S. typhimurium*.

(i) Both the *samAB* and the *umuDC*$_{ST}$ operons might be very poorly expressed when they are in a single-copy state, i.e., on the chromosome or on the 60-MDa cryptic plasmid.

(ii) Some factor(s) necessary for functions of the *samAB* and the *umuDC*$_{ST}$ operons might be missing or inactivated in *S. typhimurium*. It has been shown that the RecA protein and the heat shock-regulated chaperons GroEL and GroES are required for SOS mutagenesis in *E. coli* (9, 35). If such proteins of *S. typhimurium* have the variations which leave the proteins specifically inactive for the products of the *samAB* and *umuDC*$_{ST}$ operons, it will show the less mutable phenotype.

(iii) Some extra factors present in *S. typhimurium* but not in *E. coli* might suppress the ability of the *samAB* and

umuDC$_{ST}$ operons to promote UV mutagenesis. One of the best candidates is the 60-MDa cryptic plasmid present in *S. typhimurium*. In fact, the cryptic plasmid influences the phenotypes of host strains in several ways (42, 55). Thus, it is possible that the product(s) of a gene(s) present in the cryptic plasmid directly or indirectly suppresses the ability of the two *umuDC* operons of *S. typhimurium*.

(iv) The products of the *samAB* operon and the *umuDC*$_{ST}$ operon might titrate out each other, leading to the less mutable phenotype of *S. typhimurium*. Biochemical studies of the *E. coli umuDC* gene products indicate that UmuC can form a complex with a homodimer of UmuD′ (64). UmuD also forms a homodimer. Recently, Battista et al. provided the evidence that UmuD and UmuD′ preferentially form heterodimers, suggesting that intact UmuD is a dominant inhibitor of UmuD′-dependent mutagenesis (5). By analogy to this, two homologous but different UmuD-like proteins (or UmuD′-like proteins) encoded by the *samAB* and *umuDC*$_{ST}$ operons might form an inactive heterodimer when the two operons reside in the same cell.

A number of bacteria, such as *Haemophilus influenzae* (3), *Proteus mirabilis* (17), *Deinococcus radiodurans* (56), and *Streptococcus pneumoniae* (13), are either less mutable or nonmutable with UV and in this respect resemble *S. typhimurium* (30, 45, 48, 59). As shown in this study, the less-UV-mutable or UV-nonmutable phenotype does not necessarily mean a loss or inactivation of *umuDC* genes. On the contrary, such phenotypes may suggest that they have mechanisms to suppress or modify the function of their *umuDC*-like genes. Our results indicated that *S. typhimurium* has two sets of *umuDC* operons, which may be involved in the poor mutability of *S. typhimurium*. A survey of *umuDC*-like genes and their regulation mechanisms in a variety of organisms could facilitate our understanding of the origins and functions of *umuDC* genes.

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