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Roles of *Salmonella typhimurium umuDC* and *samAB* in UV Mutagenesis and UV Sensitivity

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Expression of the *umuDC* operon is required for UV mutagenesis and most chemical mutagenesis in *Escherichia coli*. The closely related species *Salmonella typhimurium* has two sets of *umuDC*-like operons; the *samAB* operon is located in a 60-MDa cryptic plasmid, while the *S. typhimurium umuDC* (*umuDC*$_{ST}$) operon resides in a chromosome. The roles of these two *umuDC*-like operons in UV mutagenesis and UV sensitivity of *S. typhimurium* were investigated. A pBR322-derived plasmid carrying the *samAB* operon more efficiently restored UV mutability to a *umuD44* strain and a *umuC122*::Tn*5* strain of *E. coli* than a plasmid carrying the *umuDC*$_{ST}$ operon did. When the *umuDC*$_{ST}$ operon was specifically deleted from the chromosome of *S. typhimurium* TA2659, the resulting strain was not UV mutable and was more sensitive to the killing effect of UV irradiation than the parent strain was. Curing of the 60-MDa cryptic plasmid carrying the *samAB* operon did not influence the UV mutability of strain TA2659 but did increase its resistance to UV killing. A pSC101-derived plasmid carrying the *samAB* operon did not restore UV mutability to a *umuD44* strain of *E. coli*, whereas pBR322- or pBluescript-derived plasmids carrying the *samAB* operon efficiently did restore UV mutability. We concluded that the *umuDC*$_{ST}$ operon plays a major role in UV mutagenesis in *S. typhimurium* and that the ability of the *samAB* operon to promote UV mutagenesis is strongly affected by gene dosage. Possible reasons for the poor ability of *samAB* to promote UV mutagenesis when it is present on low-copy-number plasmids are discussed.

In *Escherichia coli*, mutagenesis by UV irradiation and most chemicals requires the expression of the *umuDC* operon (17, 41, 42, 48). The *umuDC* operon is located at about 26 min on the *E. coli* chromosome and encodes 15.1- and 47.7-kDa proteins (12, 17, 18, 30, 36). Expression of the *umuDC* operon, as well as other analogous operons, such as the *mucAB* and *impAB* operons (23, 24, 30), is regulated as a part of the SOS response of *E. coli* (1, 12, 22, 43, 48–50), in which an activated form of RecA mediates the cleavage of repressor protein LexA (20, 21, 38). The activated form of RecA also mediates the cleavage of UmuD (7, 28, 35), which exhibits homology with LexA and repressors of bacteriophages λ, 434, P22, and φ80 (4, 11, 30). The resulting carboxy-terminal fragment of UmuD, UmuD′, is necessary and sufficient for the role of UmuD in UV mutagenesis (28). Recent biochemical evidence has suggested that UmuC forms a complex with a homodimer of UmuD′ (4, 52). The heat shock proteins GroEL and GroES interact with UmuC to stabilize it prior to complex formation (8, 9). Although various models have been proposed, the exact biochemical function of the UmuD′-UmuD′-UmuC complex remains unclear (3, 6, 15, 52).

In a previous paper, we demonstrated that the closely related species *Salmonella typhimurium* has two sets of *umuDC*-like operons, *S. typhimurium umuDC* (*umuDC*$_{ST}$) and *samAB* (29). The *umuDC*$_{ST}$ operon was cloned by Thomas et al. and Smith et al. as the DNA which could hybridize with the *E. coli umuDC* operon (39, 40, 45, 46). The *umuDC*$_{ST}$ and *E. coli umuDC* operons are 71% homologous at the nucleotide level (29, 40, 45). The *umuDC*$_{ST}$ operon is located at 40 min on the chromosome of *S. typhimurium* (19). The *samAB* operon was cloned in our laboratory as the genes of *S. typhimurium* which could restore UV mutability to a *umuD44* strain and a *umuC122*::Tn*5* strain of *E. coli* (29). The *samAB* operon is 40% divergent from the *umuDC*$_{ST}$ operon at the nucleotide level and exhibits higher levels of similarity to the *impAB* operon of plasmid TP110 than the *mucAB*, *umuDC*$_{ST}$, and *E. coli umuDC* operons do (29, 34). The *samAB* operon is located in a 60-MDa cryptic plasmid (29), which is commonly present in *S. typhimurium* LT2 and its derivatives (26, 31).

In order to clarify the roles of the *umuDC*$_{ST}$ and *samAB* operons in UV mutability and UV sensitivity of *S. typhimurium*, we created pBR322-derived plasmids carrying the *umuDC*$_{ST}$ or *samAB* operon and examined the ability of these plasmids to restore UV mutability to a *umuD44* strain (strain GW3200) and a *umuC122*::Tn*5* strain (strain GW2100) of *E. coli*. We also created derivatives of *S. typhimurium* TA2659 in which either the *umuDC*$_{ST}$ operon or the 60-MDa cryptic plasmid carrying *samAB* was deleted, and we compared the levels of UV mutability and UV sensitivity of these derivatives. We found that the *umuDC*$_{ST}$ operon plays a major role in UV mutagenesis in *S. typhimurium* and that the ability of the *samAB* operon to promote UV mutagenesis is strongly influenced by gene dosage.

MATERIALS AND METHODS

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

Media. Luria-Bertani broth and agar (27) were used for routine bacterial cultures and were supplemented, when

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Description	Source
E. coli strains		
GW3200	Same as AB1157, but has *umuD44*	G. C. Walker
GW2100	Same as AB1157, but has *umuC122::*Tn*5*	G. C. Walker
S. typhimurium strains		
TA2659	Same as LT2, but has Δ(*gal chl uvrB bio*) *rfa* and *hisG428*	B. N. Ames
YG5120	Same as TA2659, but its $umuDC_{ST}$ genes are replaced by a kanamycin resistance gene	This study
5046	Same as TA2659, but the 60-MDa cryptic plasmid is cured	This study
Plasmids		
pYQ122[a]	Same as 60-MDa cryptic plasmid, but has Tn*5* insertion	S. Y. Murayama
pSE117[b]	Derivative of pBR322 with the *umuDC* operon of *E. coli*	G. C. Walker
pGW2101[b]	Derivative of pZ150 with the *umuDC* operon of *E. coli*	G. C. Walker
pYA2027[b]	Same as pACYC184, but has a 3.9-kb *Sau*3A fragment carrying the *par* region at its *Bam*HI site	R. Curtiss III
pYA2028[b]	Derivative of pUC18 with the *par* region from pYA2027	R. Curtiss III
pYG8020	Same as pBluescript KS^+, but has a 3.2-kb *Eco*RV-*Eco*RV fragment carrying the *samAB* operon	T. Nohmi
pYG8020-74[b]	Same as pYG8020, but its *samB* gene is deleted	T. Nohmi
pYG8031	Same as pBR322, but its *Hin*dIII-*Sal*I region is replaced by a 2.2-kb *Hin*dIII-*Sal*I fragment carrying the $umuDC_{ST}$ operon	This study
pYG8032	Same as pBR322, but has a 3.2-kb *Eco*RV-*Eco*RV fragment carrying the *samAB* operon at its *Pvu*II site	This study
pYG8034[c]	Same as pYG8031, but has a 3.2-kb *Eco*RV-*Eco*RV fragment carrying the *samAB* operon at its *Pvu*II site	This study
pYG8035	Same as pYG8034, but the direction of the 3.2-kb DNA is opposite	This study
pYG8037	Same as pBluescript KS^+, but has a 0.9-kb *Sal*I-*Xho*I fragment carrying the $umuD_{ST}$ gene at its *Sal*I site	This study
pYG8039	Same as pSE101, but has a 2.2-kb blunt-ended *Hin*dIII-*Sal*I fragment carrying the $umuDC_{ST}$ operon at its *Hpa*I site[d]	This study
pYG8040	Same as pSE101, but has a 3.2-kb *Eco*RV-*Eco*RV fragment carrying the *samAB* operon at its *Hpa*I site[d]	This study
pYG8041	Same as pYG8030, but its 1.3-kb *Sac*II-*Sac*II region is replaced by a 1.3-kb fragment carrying a kanamycin resistance gene	This study

[a] Tn*5* is inserted in the largest *Hin*dIII fragment (fragment H1) of the 60-MDa cryptic plasmid. A restriction map of the plasmid is shown in reference 29.
[b] For detailed information about plasmids see the following references: pSE117, reference 12; pGW2101, reference 28; pYA2027 and pYA2028, reference 47; pYG8020-74, reference 29.
[c] The direction of the 3.2-kb insertion DNA is the same as the direction of the insertion DNA of pYG8032.
[d] pSE101 is a derivative of pSC101 with a kanamycin resistance gene (12).

necessary, with 50 μg of ampicillin per ml or 25 μg of kanamycin per ml. Semienriched-medium agar plates and Vogel-Bonner medium agar plates were used for a UV-induced reversion assay for *E. coli argE3* and an assay to detect reversion of *hisG428* to His^+ in *S. typhimurium* TA2659, respectively (29).

Deletion of the $umuDC_{ST}$ operon of *S. typhimurium* TA2659. Plasmid pYG8030 carrying the $umuDC_{ST}$ operon was digested with *Sac*II and then blunt ended by using a DNA-blunting kit (Takara Shuzo Co., Kyoto, Japan). The *Sac*II sites are located at codon 30 of the *umuD* gene ($umuD_{ST}$) and at codon 309 of the *umuC* gene ($umuC_{ST}$) in *S. typhimurium*. The DNA was ligated with the 1.3-kb DNA fragment carrying the kanamycin resistance gene prepared from plasmid pUC-4K (Pharmacia, Inc., Piscataway, N.J.). The resulting plasmid, designated pYG8041, had the kanamycin resistance gene in a middle region of the $umuDC_{ST}$ operon. The direction of transcription of the kanamycin resistance gene of pYG8041 is opposite the direction of transcription of $umuDC_{ST}$. Plasmid pYG8041 was introduced into *S. typhimurium* TA1538 for modification of DNA. The modified plasmid DNA was digested with *Eco*RI plus *Bam*HI, and the resulting 4-kb DNA fragment containing the kanamycin resistance gene was purified. After the DNA was self-ligated with T4 DNA ligase, the treated DNA was introduced into *S. typhimurium* TA2659 by electroporation (Bio-Rad Laboratories, Richmond, Calif.), and kanamycin-resistant colonies were selected. Mini-prep DNA was prepared from the surviving cells to make sure that there was no plasmid DNA (5). The total DNA prepared from cultures of the kanamycin-resistant colonies was used for a Southern blot analysis in which a 2.2-kb DNA fragment containing $umuDC_{ST}$ or the 1.3-kb DNA fragment carrying the kanamycin resistance gene was used as the DNA probe. The 2.2-kb DNA fragment containing $umuDC_{ST}$ was prepared as described previously (29).

Curing the 60-MDa cryptic plasmid of *S. typhimurium* TA2659. *S. typhimurium* TA2659 was transformed with pYQ122 carrying Tn*5*, and kanamycin-resistant colonies were selected. Luria-Bertani broth containing novobiocin (Sigma Chemical Co., St. Louis, Mo.) at a concentration of 20 μg/ml was inoculated with a kanamycin-resistant colony, and the resulting preparation was incubated overnight at 43°C. The overnight culture was spread onto Luria-Bertani agar plates without drugs, and then the plates were incubated overnight at 37°C. The kanamycin-sensitive colonies were screened by using the replica method (27). Curing of plasmid pYQ122 from the kanamycin-sensitive colonies was confirmed by subjecting the mini-prep DNA to agarose gel electrophoresis followed by ethidium bromide staining (16). Deletion of *samAB* was also confirmed by performing a Southern blot analysis in which the 3.2-kb DNA fragment

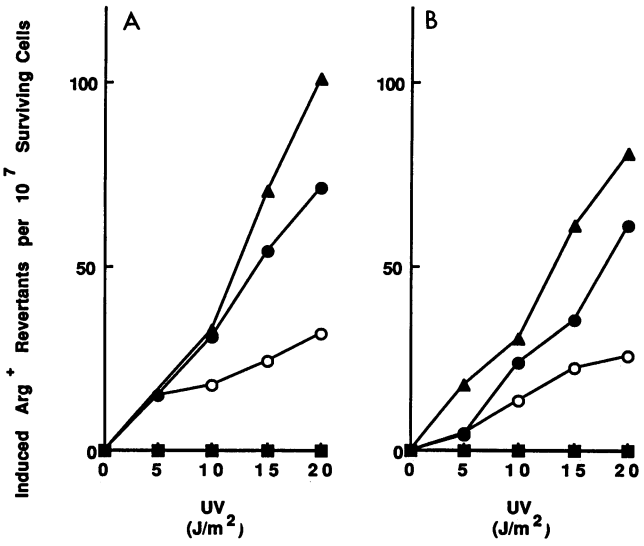

FIG. 1. Effects of pYG8031 carrying *umuDC*$_{ST}$ and pYG8032 carrying *samAB* on the frequency of Arg$^+$ reversion induced by UV irradiation in an AB1157 *umuD44* strain (A) and an AB1157 *umuC122*::Tn*5* strain (B). Symbols: ○, pYG8031 (*umuDC*$_{ST}$); ●, pYG8032 (*samAB*); ▲, pSE117 (*E. coli umuDC*); ■, pBR322.

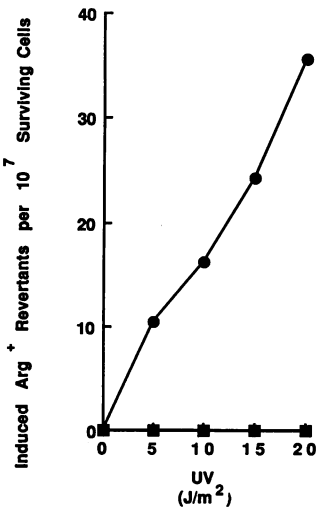

FIG. 2. Effects of pYG8037 carrying *umuD*$_{ST}$ and pYG8020-74 carrying *samA* on the frequency of Arg$^+$ reversion induced by UV irradiation in an AB1157 *umuD44* strain. Symbols: ●, pYG8037 (*umuD*$_{ST}$); ■, pYG8020-74 (*samA*).

carrying *samAB* was used as the DNA probe. The DNA fragment containing *samAB* was prepared as described previously (29).

UV mutagenesis assays. Quantitative UV mutagenesis assays with *E. coli* and *S. typhimurium* were performed as described previously (29).

RESULTS

UV mutability of *E. coli* strains harboring pBR322-derived plasmids carrying the *umuDC*$_{ST}$ operon or the *samAB* operon. In order to compare the abilities of the *umuDC*$_{ST}$ and *samAB* operons to promote UV mutagenesis, we introduced pBR322-derived plasmids carrying either the *umuDC*$_{ST}$ operon (pYG8031) or the *samAB* operon (pYG8032) into a *umuD44* strain and a *umuC122*::Tn*5* strain of *E. coli* and compared the levels of UV mutability. We also introduced plasmid pSE117 carrying the *E. coli umuDC* operon and pBR322 into the strains as controls (Fig. 1). The *E. coli* strains harboring pSE117 exhibited the highest levels of UV mutability. The strains harboring plasmid pYG8032 carrying the *samAB* operon exhibited higher levels of UV mutability than the strains harboring plasmid pYG8031 carrying the *umuDC*$_{ST}$ operon; the strains harboring pYG8032 exhibited 20 to 30% lower UV mutability than the strains harboring plasmid pSE117, while the strains harboring pYG8031 exhibited about 70% lower mutability. The strains harboring plasmid pBR322 did not exhibit any UV mutability even at a UV dose of 20 J/m^2.

To examine whether the *umuD*$_{ST}$ gene or the *samA* gene can complement the *umuD44* mutation of *E. coli*, we introduced pBluescript-derived plasmids carrying either the *umuD*$_{ST}$ gene (pYG8037) or the *samA* gene (pYG8020-74) into a *umuD44* strain and determined the levels of UV mutability of the transformants. We found that plasmid pYG8037 efficiently restored UV mutability to a *umuD44* strain, while plasmid pYG8020-74 did not (Fig. 2). Thomas et al. (45) reported that a pBR322-derived plasmid carrying the *umuD*$_{ST}$ gene restored UV mutability to a *umuD77* strain of *E. coli*. These findings suggested that the *umuD*$_{ST}$ gene is functionally more similar to the *umuD* gene of *E. coli* than the *samA* gene is.

Deletion of the *umuDC*$_{ST}$ operon and curing of the 60-MDa cryptic plasmid of *S. typhimurium* TA2659. In order to assess the exact roles of the *umuDC*$_{ST}$ and *samAB* operons in UV sensitivity and UV mutagenesis in *S. typhimurium*, we created derivatives of *S. typhimurium* TA2659 in which either the *umuDC*$_{ST}$ operon or the 60-MDa cryptic plasmid carrying *samAB* was deleted.

The *umuDC*$_{ST}$-deficient derivative, a strain YG5120 derivative, was created by replacing the 1.3-kb middle region of the *umuDC*$_{ST}$ operon with the kanamycin resistance gene (Fig. 3). To confirm the gene replacement, multiple restriction enzyme digests of the total DNA of strain YG5120 were

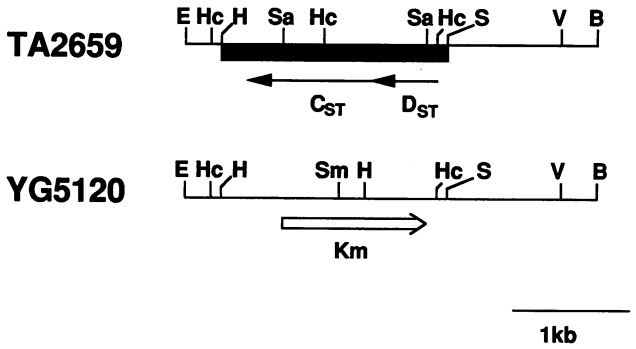

FIG. 3. Partial restriction maps around the *umuDC*$_{ST}$ operon of strain TA2659 and the corresponding region of strain YG5120. The arrows beneath the strain TA2659 map indicate the direction of transcription of *umuDC*$_{ST}$. The arrow beneath the strain YG5120 map indicates the direction of transcription of the kanamycin resistance gene. The solid bar indicates the position of a 2.2-kb DNA fragment that was used as a probe. Abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; S, *Sal*I; Sa, *Sac*II; Sm, *Sma*I; V, *Eco*RV.

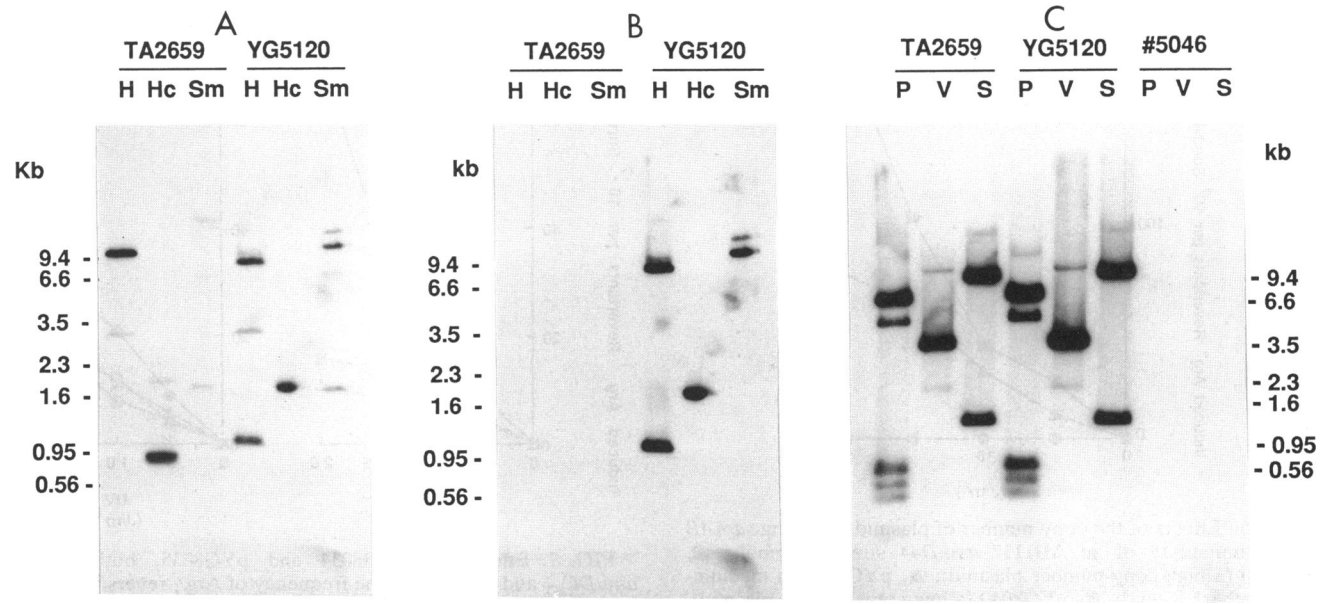

FIG. 4. Southern hybridization analysis of the DNAs of *S. typhimurium* TA2659, YG5120 ($\Delta umuDC_{ST}$), and 5046 (Δ60-MDa cryptic plasmid) performed with probes carrying $umuDC_{ST}$ (A), a kanamycin resistance gene (B), or *samAB* (C). Abbreviations: H, *Hin*dIII; Hc, *Hin*cII; Sm, *Sma*I; P, *Pst*I; V, *Eco*RV; S, *Sal*I.

subjected to a Southern blot analysis. When the 2.2-kb DNA containing the $umuDC_{ST}$ operon was used as a probe, different banding patterns were observed for the digests of the DNAs of strains YG5120 and TA2659 (Fig. 4A). Typically, an intense band at 9.6 kb was observed in a *Hin*dIII digest of strain TA2659 DNA, while two intense bands at 1.3 and 8.3 kb were observed in a *Hin*dIII digest of strain YG5120 DNA. The kanamycin resistance gene has a single *Hin*dIII site, while the $umuDC_{ST}$ operon does not have this site. When the kanamycin resistance gene was used as a probe, only strain YG5120 lanes contained the positive bands, and the banding pattern was basically the same as that observed when $umuDC_{ST}$ was used as the DNA probe (Fig. 4B). When the *samAB* operon was used as the probe, the digests of strain YG5120 DNA produced exactly the same banding pattern as the digest of strain TA2659 DNA, suggesting that the *samAB* operon was not disrupted during gene replacement (Fig. 4C).

Strain TA2659 harboring plasmid pYQ122 carrying the Tn*5* insertion was incubated overnight with novobiocin at 43°C and then single colonies were isolated; 42 of the resulting 364 colonies (11.5%) exhibited sensitivity to kanamycin. When mini-prep DNAs were prepared from these kanamycin-sensitive colonies and the DNAs were examined by agarose gel electrophoresis followed by ethidium bromide staining, no high-molecular-weight plasmid DNA was observed. One such plasmid-free colony was designated strain 5046, and the total DNA of this organism was subjected to a Southern blot analysis. No hybridization bands were observed in the restriction enzyme digests of strain 5046 when the *samAB* operon was used as the DNA probe (Fig. 4C). These results suggested that strain 5046 contained no cryptic plasmid and also that the *samAB* operon was not integrated into chromosome DNA as the cryptic plasmid was cured.

***umuDC*$_{ST}$ operon plays a major role in UV mutagenesis in *S. typhimurium*.** The sensitivities of strains YG5120 and 5046 to the killing and mutagenic effects of UV irradiation were compared with the sensitivity of parent strain TA2659. Strain YG5120 was more sensitive to the killing action of UV irradiation than strain TA2659 was, whereas strain 5046 exhibited resistance to UV irradiation and a 100% survival rate even at a UV dose of 3 J/m^2 (Fig. 5A). In contrast, strain YG5120 exhibited a nonmutable phenotype to UV irradiation, while strain 5046 exhibited almost the same level of mutability or even a higher level of mutability than the parent strain did (Fig. 5B). These results suggested that the $umuDC_{ST}$ operon plays a major role in UV mutagenesis in *S. typhimurium* TA2659, while both the $umuDC_{ST}$ operon and the cryptic plasmid carrying the *samAB* operon affect the UV sensitivity of this organism.

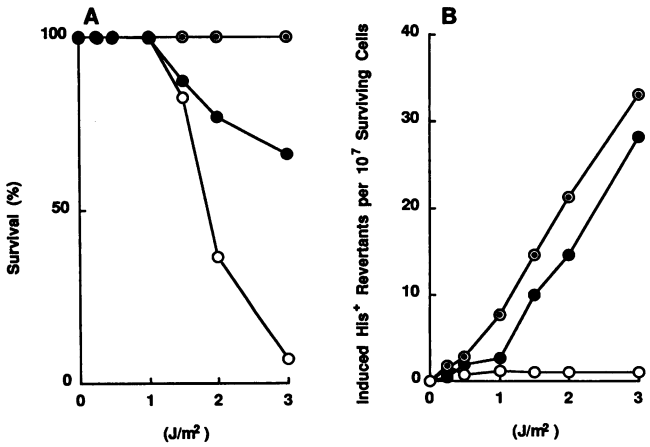

FIG. 5. Effects of deletion of $umuDC_{ST}$ and curing of the 60-MDa cryptic plasmid carrying *samAB* on UV sensitivity (A) and UV mutability (B) of *S. typhimurium* TA2659. Symbols: ●, strain TA2659; ○, strain YG5120 ($\Delta umuDC_{ST}$); ⊙, strain 5046 (Δ60-MDa cryptic plasmid).

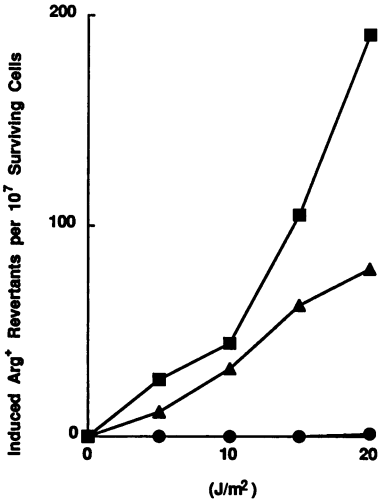

FIG. 6. Effects of the copy number of plasmids carrying *samAB* on UV mutability of an AB1157 *umuD44* strain. Symbols: ■, pYG8020 (a high-copy-number plasmid); ▲, pYG8032 (a medium-copy-number plasmid); ●, pYG8040 (a low-copy-number plasmid).

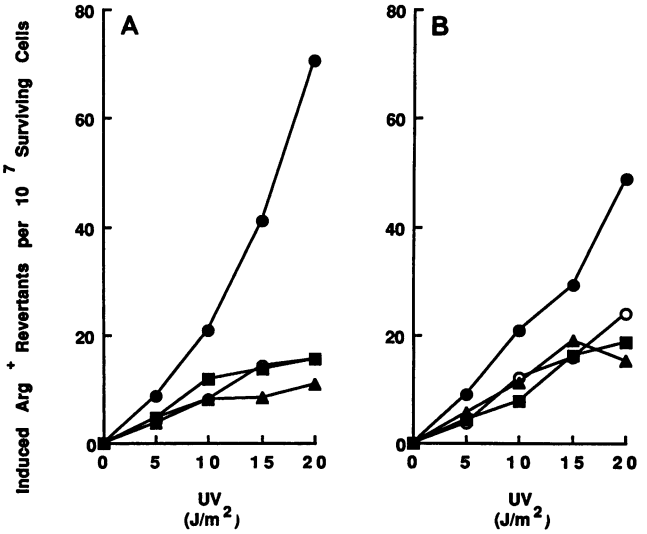

FIG. 7. Effects of pYG8034 and pYG8035, both carrying $umuDC_{ST}$ and *samAB*, on the frequency of Arg^+ reversion induced by UV irradiation in an AB1157 *umuD44* strain (A) and an AB1157 *umuC122*::Tn*5* strain (B). Symbols: ○, pYG8031 ($umuDC_{ST}$); ●, pYG8032 (*samAB*); ▲, pYG8034 ($umuDC_{ST}$ and *samAB*); ■, pYG8035 ($umuDC_{ST}$ and *samAB*).

Ability of the *samAB* operon to promote UV mutagenesis is strongly affected by gene dosage. It was puzzling that the *samAB* operon on the cryptic plasmid did not seem to significantly contribute to the UV mutability of *S. typhimurium* TA2659 (Fig. 5B), whereas the pBR322-derived plasmid carrying the *samAB* operon efficiently restored UV mutability to the *umuDC*-deficient strains of *E. coli* (Fig. 1). To investigate how copy number affects the ability of *samAB* to promote UV mutagenesis, we introduced low-, medium-, and high-copy-number plasmids carrying the *samAB* operon into a *umuD44* strain of *E. coli* and compared the resulting UV mutabilities. We found that mutability depended on the copy number of the plasmids carrying the *samAB* operon (Fig. 6). The strain harboring the pBluescript-derived plasmid carrying the *samAB* operon (pYG8020) exhibited a very high level of mutability, which was even higher than the level of mutability of the strain harboring pSE117 or pGW2101, a high-copy-number plasmid carrying the *E. coli umuDC* operon (28). In contrast, the strain harboring the pSC101-derived plasmid carrying the *samAB* operon (pYG8040) was virtually not mutable by UV irradiation. Unlike the *samAB* operon, a *umuD44* strain harboring a pSC101-derived plasmid carrying the $umuDC_{ST}$ operon (pYG8039) was mutable by UV irradiation; we observed 16.7 induced Arg^+ revertants per 10^7 surviving cells at a UV dose of 20 J/m^2. These results suggested that the ability of the *samAB* operon to promote UV mutagenesis is strongly affected by the gene dosage.

***samAB* genes are apparently recessive to $umuDC_{ST}$ genes in terms of UV mutagenesis.** In order to investigate the reason for the poor ability of *samAB* to promote UV mutagenesis when it is on low-copy-number plasmids, we introduced pBR322-derived plasmids carrying both operons (pYG8034 and pYG8035) into a *umuD44* strain and a *umuC122*::Tn*5* strain of *E. coli* and compared the levels of UV mutability with the levels for the strains harboring plasmids carrying either $umuDC_{ST}$ alone or *samAB* alone (Fig. 7). In both backgrounds, the strains harboring the plasmids carrying both operons exhibited almost the same level of UV mutability as the strains harboring pYG8031 carrying $umuDC_{ST}$ alone did. Plasmids pYG8034 and pYG8035 have the *samAB* operon in opposite orientations, and they exhibited almost the same level of UV mutability. These results suggested that the *samAB* genes are recessive to the $umuDC_{ST}$ genes in terms of UV mutagenesis when the two operons are present on the same multi-copy-number plasmid.

***samAB* operon is located near the *par* region of the 60-MDa cryptic plasmid.** Tinge and Curtiss reported that the restriction map of the *par* region of the 60-MDa cryptic plasmid (47). The map was in part similar to the map of the region containing the *samAB* operon (29). The *par* region appears to be involved in partitioning of the cryptic plasmid (47). To examine the possibility that the *samAB* operon is located near the *par* region, the DNAs of plasmids pYA2027 and pYA2028, both of which carried the *par* region, were subjected to a Southern hybridization analysis in which the *samAB* operon was used as the probe. Both of the plasmids strongly hybridized with the probe DNA carrying the *samAB* sequence (Fig. 8). The electrophoretic mobilities of all of the positive bands corresponded to the electrophoretic mobilities of the plasmid DNA bands visualized by ethidium bromide staining on the agarose gel, suggesting that the positive bands were not due to contaminated cryptic plasmid DNA but were due to plasmids pYA2027 and pYA2028 themselves. Thus, we concluded that the *samAB* operon resides near the *par* region of the cryptic plasmid (Fig. 9).

DISCUSSION

In a previous paper, we demonstrated that *S. typhimurium* has two *umuDC*-like operons, the $umuDC_{ST}$ operon and the *samAB* operon (29). This finding prompted us to investigate the roles of the *umuDC*-like operons in the UV mutability and UV sensitivity of *S. typhimurium*. Of the two *umuDC*-like operons of *S. typhimurium*, the *samAB* operon was more effective in restoring UV mutagenesis to *umuDC*-deficient strains of *E. coli* than the $umuDC_{ST}$ operon when the operons resided on pBR322 vectors (Fig. 1). This ap-

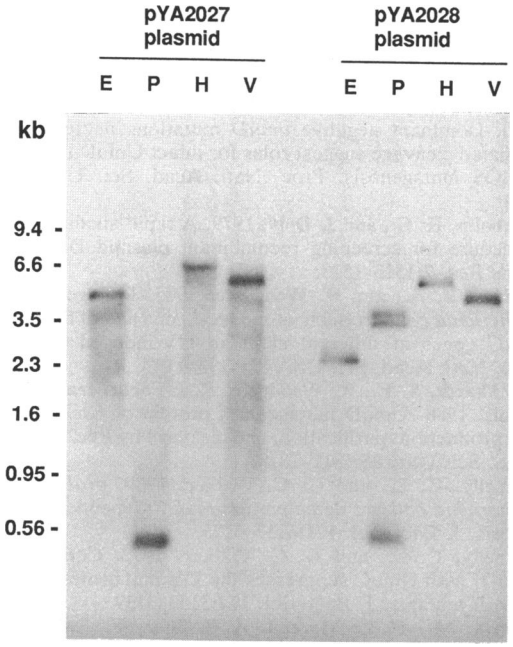

FIG. 8. Southern hybridization analysis of pYA2027 and pYA2028 performed with the probe carrying *samAB*. Both pYA2027 and pYA2028 contain the *par* region of the 60-MDa cryptic plasmid (47). The DNA fragment containing *samAB* used for the probe was prepared as described previously (29). Abbreviations: E, *Eco*RI; P, *Pst*I; H, *Hin*dIII; V, *Eco*RV.

pears to be reasonable since the *samAB* operon was cloned as genes on the pBR322 vector, which can efficiently complement the *umuD44* mutation and the *umuC122*::Tn*5* mutation of *E. coli* (29). The $umuDC_{ST}$ operon was cloned as DNA which can hybridize with the *E. coli umuDC* sequence (39, 40, 45, 46). However, even the strains harboring the plasmid carrying *samAB* exhibited lower levels of UV mutability than the strains harboring plasmid pSE117 carrying the *E. coli umuDC* operon did. It is known that *S. typhimurium* exhibits a weaker mutagenic response to UV irradiation and some chemical mutagens than *E. coli* does (14, 25, 32, 37). The poorly UV-mutable phenotype of *S. typhimurium* could be due to the poor abilities of $umuDC_{ST}$ and *samAB* to promote UV mutagenesis.

The pBluescript-derived plasmid carrying the *samA* gene alone did not restore UV mutability to a *umuD44* strain of *E. coli*, while the plasmid carrying the $umuD_{ST}$ gene alone

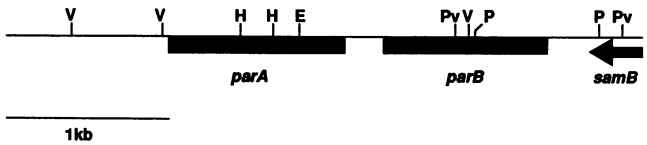

FIG. 9. Partial restriction map of the insertion DNA of pYA2027 and the positions of *parA, parB,* and *samB*. The positions of *parA* and *parB* are given in reference 47, and the position of *samB* was determined by Southern hybridization (Fig. 8) and by comparing restriction maps. The arrow indicates the direction of transcription of *samAB*. The restriction map of *samAB* and the position of *samAB* in a whole 60-MDa cryptic plasmid are given in reference 29. Abbreviations: E, *Eco*RI; P, *Pst*I; Pv, *Pvu*II; H, *Hin*dIII; V, *Eco*RV.

efficiently did (Fig. 2). This suggests that the *samB* gene is not replaced by the *E. coli umuC* gene and that the *samA* gene needs its cognate partner *(samB)* for UV mutagenesis. Similar results have been reported for the *mucAB* and *impAB* operons; neither *mucA* nor *impA* complements the *umuD* mutation of *E. coli* (30, 34). The *samAB* operon exhibits the higher levels of similarity to the *impAB* operon at the amino acid level than the *E. coli umuDC, mucAB,* and $umuDC_{ST}$ operons do (29, 34). In this respect, it might be interesting to determine whether the combination of *impA* and *samB*, as well as the combination of *samA* and *impB*, can result in a mutagenic response to UV irradiation.

Despite the presence of *samAB*, the specific deletion of $umuDC_{ST}$ abolished the UV mutability of *S. typhimurium* TA2659 (Fig. 5). Curing of the cryptic plasmid carrying the *samAB* operon did not substantially influence UV mutability. These results suggest that the $umuDC_{ST}$ operon plays a major role in UV mutagenesis of *S. typhimurium* TA2659 and that the *samAB* operon on the cryptic plasmid does not substantially contribute to UV mutability. Similar results have been reported by Koch et al., who found that UV mutability of *S. typhimurium* LT2 was eliminated despite the presence of *samAB* when any of the $umuDC_{ST}$ genes was altered (19). Our results also indicated that gene dosage is very important for the ability of *samAB* to promote UV mutagenesis in *E. coli* and probably in *S. typhimurium* (Fig. 6). The fact that the gene products cannot promote UV mutagenesis when the genes are on a low-copy-number plasmid appears to be unique to the *samAB* operon since low-copy-number plasmids carrying the *E. coli umuDC, mucAB,* or $umuDC_{ST}$ operon clearly restored UV mutability to a *umuD44* strain of *E. coli.* In contrast to UV mutability, not only the $umuDC_{ST}$ operon but also the cryptic plasmid carrying the *samAB* operon seems to play an important role in the UV sensitivity of *S. typhimurium* TA2659 (Fig. 5). In order to determine the role of *samAB* more precisely, specific deletion of the *samAB* operon will be required. In contrast to our results, Koch et al. reported that neither the alteration of $umuDC_{ST}$ nor the curing of the cryptic plasmid affected the UV sensitivity of *S. typhimurium* LT2 (19). *S. typhimurium* TA2659 is a *uvrB* deletion derivative of *S. typhimurium* LT2. It remains to be determined whether the different status of excision repair explains the difference between our results and the results of Koch et al.

Why are the products of the *samAB* operon unable to promote UV mutagenesis when the genes are on low-copy-number plasmids? It has been reported that *samAB* transcripts are observable after *S. typhimurium* harboring the cryptic plasmid is treated with mitomycin C (19). We demonstrated previously by using a maxicell technique that the efficiency of expression of *samAB* is comparable with the efficiency of expression of *E. coli umuDC* (29). Therefore, we ruled out the possibility that the *samAB* genes are not expressed when they are on the cryptic plasmid even if *S. typhimurium* is treated with DNA-damaging agents. The deduced amino acid sequence of SamA includes Ala-24 and Gly-25 residues at the putative cleavage site, as in MucA and ImpA (4, 23, 30). This sequence also includes Ser-61 and Lys-98 residues, which probably play important roles in the autocatalytic cleavage reaction (28, 38). Thus, it seems unlikely that the SamA protein is deficient in RecA-dependent cleavage during SOS induction. $UmuD_{ST}$ is cleaved in a RecA-dependent fashion (51).

samAB is apparently recessive to $umuDC_{ST}$ in terms of UV mutagenesis when the two operons are present on the same pBR322 vector (Fig. 7). These results led us to postu-

late that the products of *samAB* and $umuDC_{ST}$ are competitive for some critical factor(s) for UV mutagenesis and that the products of *samAB* have much lower affinities for the factor than the products of $umuDC_{ST}$ do. It has been reported that RecA plays a third role in UV mutagenesis in addition to mediating the cleavage of LexA and UmuD (10, 28). Although the exact nature of this third role of RecA is not known, one of the interesting possibilities is that RecA binds to the UmuD'C complex and guides it to the DNA lesions (2, 44). In fact, UmuC binds tightly to a RecA affinity column, and UmuD' also binds to such a column when UmuC is present (13). By analogy, we postulate that the products of *samAB* may have much lower affinity for RecA of *E. coli* and RecA of *S. typhimurium* than the products of $umuDC_{ST}$ do. If this is the case, the concentrations of SamAB in normal *S. typhimurium* cultures would be too low to allow them to interact with RecA of *S. typhimurium*. Once the products of *samAB* are overproduced and allowed to interact with RecA, they should be able to promote UV mutagenesis more efficiently than the products of $umuDC_{ST}$ do. At this moment, however, we cannot rule out another possibility, that a defect in SamA-SamA or SamA-SamB interaction could explain the inability of the *samAB* operon to promote UV mutagenesis when it is on low-copy-number plasmids. Further investigation of the possible interaction of SamAB or $UmuDC_{ST}$ with RecA may shed light on the mechanism of UV mutagenesis in *S. typhimurium* and in *E. coli*.

A recent survey indicated that *umuDC*-like sequences or proteins are present in a wide variety of enteric bacteria (33). Interestingly, differences in UV-induced mutability of more than 200-fold have been found in different species of bacteria; some strains of *E. coli* are apparently not mutable by UV irradiation, whereas others are highly mutable. Although it has not been determined whether the low level of mutability of such bacteria is due to the poor ability of the *umuDC*-like proteins to mediate UV mutagenesis, these results suggest that the *samAB* operon may represent a group of *umuDC*-like sequences that do not substantially contribute to the UV mutability of host strains. The results described above also raise the possibility that the *umuDC*-like sequences are retained not only for mutagenesis but also for other cellular processes that remain to be identified. The *samAB* operon was mapped near the *par* region of the 60-MDa cryptic plasmid of *S. typhimurium* (Fig. 8 and 9). In order to investigate the possible role of the *samAB* operon in plasmid replication and maintenance, as well as to assess its role in UV mutagenesis and UV sensitivity more precisely, we are currently creating a specific deletion of the *samAB* operon in the cryptic plasmid of *S. typhimurium*.

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