JOURNAL OF BACTERIOLOGY, May 1991, p. 2906–2914
0021-9193/91/092906-09$02.00/0

Vol. 173, No. 9

Heterospecific Expression of Misrepair-Enhancing Activity of *mucAB* in *Escherichia coli* and *Bacillus subtilis*

HIROSHI TANOOKA,* KAZUHIKO TANAKA, AND KUMIKO SHINOZAKI

Radiobiology Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104, Japan

Received 24 October 1990/Accepted 1 March 1991

Enterobacterial plasmid genes *mucAB*, which possess error-prone repair activity, were cloned and sequenced independently of a sequence previously determined (K. L. Perry, S. J. Elledge, B. B. Mitchell, L. Marsh, and G. C. Walker, Proc. Natl. Acad. Sci. USA 82:4331–4335, 1985). The survival- and mutation-enhancing activities of *mucAB* ligated to the MLS^r promoter of a *Bacillus subtilis* plasmid in the shuttle vector pTE22R were expressed in *B. subtilis* as well as in *Escherichia coli* after mutagenic treatment. *mucAB* fragments with 5′ deletions of various lengths up to the base sequence encoding Ala-26–Gly-27, the putative RecA-mediated cleavage site of the MucA protein, showed mutation-enhancing activity for noninducible *lexA3 E. coli* when ligated to the MLS^r promoter in frame. This activity was lost by extending the deletion downstream. The formations of MucA and MucB proteins in *B. subtilis* and *E. coli* were demonstrated by Western blot (immunoblot) analysis. MucA cleavage in Rec$^+$ *B. subtilis* was observed only after treatment with an alkylating agent and was not observed in RecA$^-$ and RecE$^-$ strains, whereas in *E. coli* cleavage was observed in Rec$^+$ cells after treatment with either mitomycin C or an alkylating agent but was not detected in RecA$^-$ cells. Common activity of *B. subtilis* Rec and *E. coli* RecA in the induction of mutants is suggested.

The first transmissible antibiotic resistance factor (R factor) was discovered in *Shigella* strains of enterobacteria (57). Later, *Salmonella* R factors were shown to confer to bacteria resistance to UV irradiation (8, 24). Plasmid pKM101, a derivative of the original R46 (34), contains the *mucAB* operon (16), which consists of two genes (*mucA* and *mucB*) (42) and which enhances not only cell survival but also the induction of mutations after treatment with UV irradiation or a mutagenic chemical (56). This error-prone repair is distinguishable from error-free repair and is considered to be associated with cellular SOS-inducible functions (18, 44, 56, 58). Because of this error-prone activity, *Salmonella typhimurium* containing pKM101 has been widely used as a sensitive means of detection of environmental mutagens (31).

The nucleotide sequence of *mucAB* was first reported by Perry et al. (42), who pointed out its structural and functional homologies to those of *umuDC*, *Escherichia coli* chromosomal genes essential for induced mutagenesis (13). Homology to *mucAB* and *umuDC* has recently been found in *impAB* in TP100 (20), *samAB* in a 60-MDa cryptic plasmid (38), and chromosomal genes $umuDC_{ST}$ (48, 52) of *S. typhimurium*. These genes have a LexA repressor-binding site called the SOS box (19) in the promoter, as do other SOS-regulated genes (18). The repressed genes are derepressed by RecA-mediated cleavage of the LexA repressor (11, 17, 47), whereby RecA activated in response to formation of damage to DNA acts indirectly to stimulate self-cleavage of LexA (47). The MucA protein itself possesses a putative cleavage site (29, 42) like that in the UmuD protein (14, 42). Cleavage is thought to be associated with cellular mutagenesis mechanisms. An interesting hypothesis was recently proposed, i.e., that UmuC and the cleaved UmuD proteins form a complex with DNA lesions so that DNA polymerase can use the damaged DNA as a template in *E. coli* (60). However, cleavage upon mutagenic treatment has been demonstrated only for the UmuD protein (4, 37, 46).

We are interested in whether a common mutagenic pathway like the RecA-LexA pathway of *E. coli* exists in *Bacillus subtilis* and other bacteria. There are reports of the presence of DNA damage-inducible genes in *B. subtilis* (21, 22). Moreover, the Rec protein activity of *B. subtilis* is similar to that of the *E. coli* RecA protein (7, 22, 23). Expression of introduced *mucAB* in *Haemophilus influenzae* (1, 49), and to a lesser extent in yeast (43) and mouse cells (27), has been shown.

In this paper, we report further characterization of the *mucAB* activity in *E. coli* and its expression in *B. subtilis*. Previously, we described the oncogene-like activity of *mucAB* ligated to a mammalian promoter in mouse BALB 3T3 cells (53).

MATERIALS AND METHODS

Molecular cloning methods. The methods used for molecular cloning were as described by Maniatis et al. (26).

Bacteria. *E. coli* HB101 (*recA13*) was used as a host for cloning *mucAB* and its deletions. The *E. coli* strains employed for mutation tests and protein analysis were WP2s (*uvrA155 trpE65*) (59), CM611 (*lexA102 uvrA155 trpE65*) (3), ZA81 (*lexA3 uvrA6 his-4 thr-1 leu-6 proA2 thi-1 ilv-325*) (40a) with *lexA3* from DM49 (35), GW2730 (*lexA71*::Tn*5 recA441* F$^-$ *thr-1 leu-6 his-4 argE3 galK2 strA31 ilv sfiA11 lacU169*) (15, 28), and WP100 (*recA56 urvA155 trpE65*) (10). WP2s, CM611, and WP100 are *E. coli* B derivatives; ZA81 and GW2730 are K-12 derivatives; and HB101 is a hybrid of B and K-12 (2). The *B. subtilis* strains used were TKJ5211 (*uvrA10 his-101 met-101*) (51) and the alkylating agent-sensitive double mutant TKJ6951 (*ada-1 dat-1 his-101 leu lys thyAB*) defective in inducible and constitutive O^6-alkylguanine-DNA alkyltransferase (33). The His$^-$ phenotype is highly revertable by suppressor mutation (41, 51). The *recA1* and *recE4* mutations (9) of *B. subtilis* GSY1025 and GSY908 were introduced into TKJ6951 by transformation, and the

* Corresponding author.

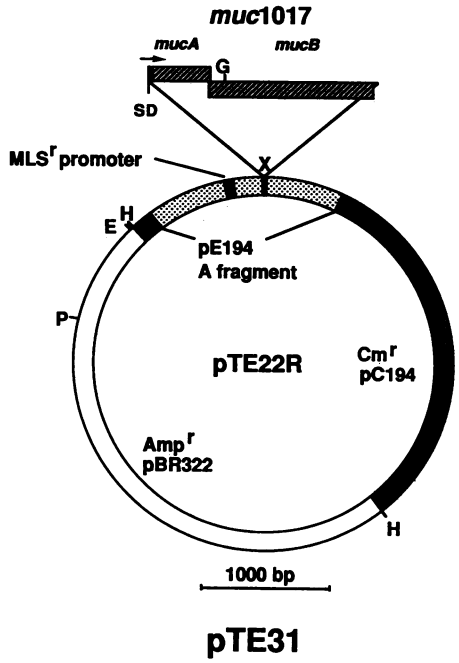

FIG. 1. Plasmid pTE31, consisting of the *E. coli-B. subtilis* shuttle vector pTE22R (pHW1-pBR322) with inserted *muc*1017. Restriction sites: E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; P, *Pst*I; X, *Xho*I.

resulting strains were named TKJ9200 and TKJ9201, respectively.

Vector plasmids. The cloning vector was pBR322, in which the *Bam*HI site blunt end ligated with an *Xho*I linker (Toyobo, Osaka, Japan) was used for cloning. pGC2 (36) with multiple cloning sites was also used. The *B. subtilis* plasmid used was pHW1 (12), which consisted of 2,910 bp of pC194 and a 1,443-bp A fragment of *Taq*I-cleaved pE194 carrying the gene conferring *B. subtilis* with resistance to macrolide, lincosamide, and streptogramin type B (MLS^r). An 8,714-bp *E. coli-B. subtilis* shuttle vector was constructed by ligation of pHW1 with pBR322 at the unique *Hin*dIII sites and named pTE22R (Fig. 1). The pTE22R site used for insertion of *mucAB* was the *Bcl*I site with an 8-bp *Xho*I linker 123 bases downstream from the initiation codon of the MLS^r gene. This part of the MLS^r gene encodes an 8.8-kDa MLS^r protein with 41 amino acids. It was designed to produce a fused protein between the 8.8-kDa MLS^r protein and the deleted MucA protein when *mucAB* with various 5′ deletions was inserted in frame.

***mucAB* and *umuDC*.** pKM101 was a gift from B. N. Ames to M. Nagao and K. Wakabayashi of the National Cancer Center Research Institute and was transferred to us. pKM101 was digested with *Acc*I according to the map of Langer et al. (16), and the 4.5-kb fragment containing *mucAB* was cloned into pBR322. The resulting plasmid, named pTE185, was further digested with *Bal* 31 from the 5′ end, and the shortened 2.3-kb fragment was cloned into pBR322 at the *Bam*HI site with *Xho*I linkers. This plasmid, pTE186, was digested with *Bal* 31 from the 5′ end, and the 3′ end of the fragment was cleaved by *Sma*I. The 1.99-kb fragment containing the whole *mucAB* operon was named *muc*364.

The nucleotide sequence of *muc*364 was determined by the method of Maxam and Gilbert (30), by using restriction sites of *Ban*I, *Bgl*II, *Bst*NI, *Dde*I, *Hin*fI, *Hin*dII, *Nci*I, *Xma*III, and *Xho*I. Chemically cleaved DNA fragments were analyzed after electrophoresis in an 8 or 20% polyacrylamide gel. The sequence of each fragment was confirmed by sequencing in both directions.

*muc*364 was inserted into the *Xho*I linker-ligated *Bam*HI site of pBR322 with *Xho*I linkers so that the 5′ end of *muc*364 was proximate to the *Eco*RI site of pBR322, and the plasmid was named pTE30. pTE30 was further digested from the *Eco*RI site with *Bal* 31 to obtain various 5′ deletions of *mucAB*. These DNA fragments were cloned into pBR322 at the *Bam*HI site with *Xho*I linkers, and the nucleotide sequence of each 5′ end was determined by the method of Maxam and Gilbert by using a 20% polyacrylamide gel (Fig. 2). The *mucAB* fragments that were in frame with the MLS^r gene with an *Xho*I linker were chosen (Fig. 2 and 3).

mucAB without the SOS box was obtained as follows. The 460-bp *Bgl*II-*Nar*I digest of *mucA*, lacking 29 bp downstream of the initiation codon of *mucA*, was ligated to a synthetic fragment of 40 bp plus 6 bases (from bp 261 to 298 [Fig. 2], with an *Xho*I site at the 5′ end and an *Nar*I site at the 3′ end), provided by S. Noguchi. The resulting 506-bp DNA fragment was cloned into pGC2. The 3′ end of this 506-bp fragment was further ligated to the 5′ end of the 1,228-bp *Bgl*II fragment of *muc*364 to yield 1,734 bp of DNA named *muc*1017. *muc*1017 with *Xho*I linkers contained the whole reading frame of *mucAB* and the Shine-Dalgarno (SD) sequence but no SOS box (Fig. 2). The reading frame of *muc*1017 coincided with that of the MLS^r gene when it was ligated to the vector pTE22R with *Xho*I linkers. This *muc*1017-pTE22R plasmid was named pTE31 (Fig. 1). pTE31 from which the pBR322 portion was removed was named pTE32 (*muc*1017-pHW1); it was used to improve the efficiency of production of MucA and MucB proteins in *B. subtilis*.

A plasmid containing *umuDC* cloned into pBR322 (pTA100) (14) was kindly provided by T. Kato.

Measurement of survival and mutation. *E. coli* and *B. subtilis* cells were grown to the late-exponential-growth phase at 37°C in Luria-Bertani broth (26) and G2 (51) media containing the required amino acids (100 μg/ml) and casein hydrolysate (500 μg/ml), respectively, supplemented with appropriate antibiotics (50 μg of ampicillin per ml and 5 μg of chloramphenicol per ml). The cells were washed twice with buffer and suspended at 10^8 cells per ml in buffer. The buffer solutions used were Davis minimal medium (6) minus glucose for *E. coli* and Spizizen minimal medium (50) minus glucose for *B. subtilis*. For UV irradiation, 5 ml of cell suspension was put into a petri dish 9 cm in diameter and irradiated with UV light (peak wave length, 254 nm) at a fluence rate of 0.165 J/m^2 per s. For chemical treatment, 1 ml of resuspended cells was incubated with chemicals for 15 min at 37°C and the cells were washed twice with buffer. Cells were plated on semienriched plates for *E. coli* (10) and *B. subtilis* (51) after the appropriate dilution for counting colonies of surviving cells, and at 10^8 cells per plate for counting colonies of His$^+$ or Trp$^+$ mutants of *E. coli* and His$^+$ *B. subtilis*. For scoring hypersensitive His$^+$ revertants of *B. subtilis*, His$^+$ colonies were counted with the help of a magnifier in areas 5 by 5 mm^2, and average numbers in three areas were multiplied by the factor 254 to give the total number of mutant colonies per 9-cm-diameter plate. The Lys$^+$ marker of *B. subtilis* TKJ6951 was also useful but gave a lower mutant yield. Colonies of surviving cells and mutants were counted after incubation at 37°C for 2 and 3 days, respectively.

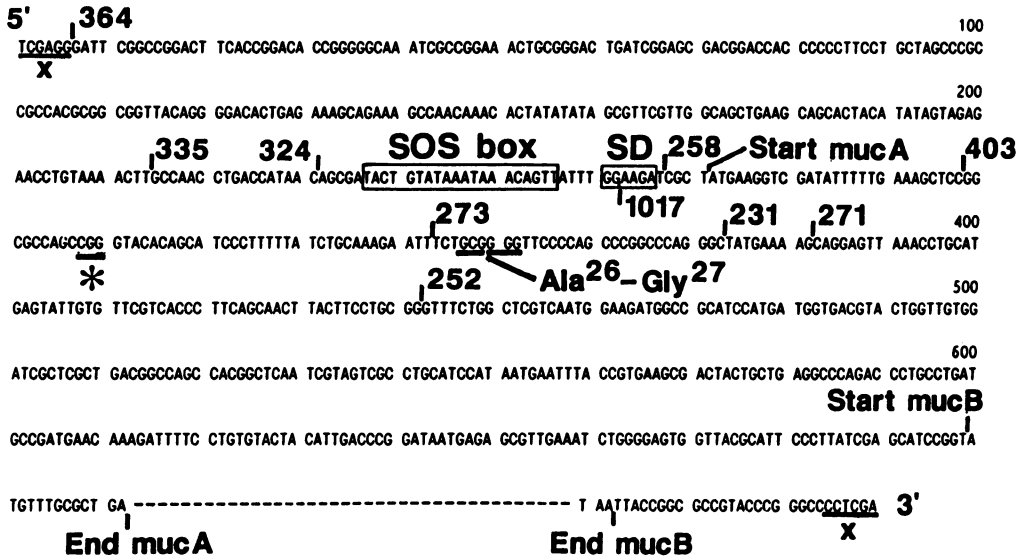

FIG. 2. Nucleotide sequence of *muc*364. Numbers indicate clone numbers of the *mucAB* fragments in which the 5′ upstream region from the indicated position is deleted. *, codon missing in the sequence reported by Perry et al. (42); ----, sequence identical to that reported by Perry et al. (42). Ala-26–Gly-27, putative cleavage site of MucA encoded by the sequence indicated; X, *Xho*I linker.

For a simpler assay of mutants, suspensions of 0.1 ml of cultured cells were plated, and 0.2 ml of 1/100 diluted (0.11 M) methylmethane sulfonate (MMS; Eastman Kodak, Rochester, N.Y.) or 0.1 mg of *N*-methyl-*N*′-nitrosoguanine (MNNG; Sigma Chemical Co., St. Louis, Mo.) per ml was spotted on a paper disk and placed on the plate (51). Colonies of auxotrophic revertants formed around the disk were an indication of the mutability of cells containing *mucAB*. Especially with noninducible *lexA3* mutants of *E. coli* ZA81 as host cells, this method gave clear-cut, all-or-none results.

Antibodies to MucA and MucB. Putative amino acid sequences of *mucA* and *mucB* gene products from the respective nucleotide sequences were obtained. The hydrophobicity and secondary structure of each protein were analyzed by the method of Chou and Fasman (5). Two candidate regions for the effective antigen from MucA, i.e., SSMEDGRIHDG DV-73 (CO18) and WGVVTHSLIEHDVCLR-146 (CO19), and two regions from MucB, i.e., TQYANQATEKLTV AT-316 (CO1A) and SPDYTTDWRSIPIATIK-424 (CO1B), were chosen, and these sequences were synthesized at Toyo Research Center (Osaka, Japan). These polypeptides were conjugated with thioglobulin mixed with Freund complete adjuvant and injected subcutaneously three times weekly in

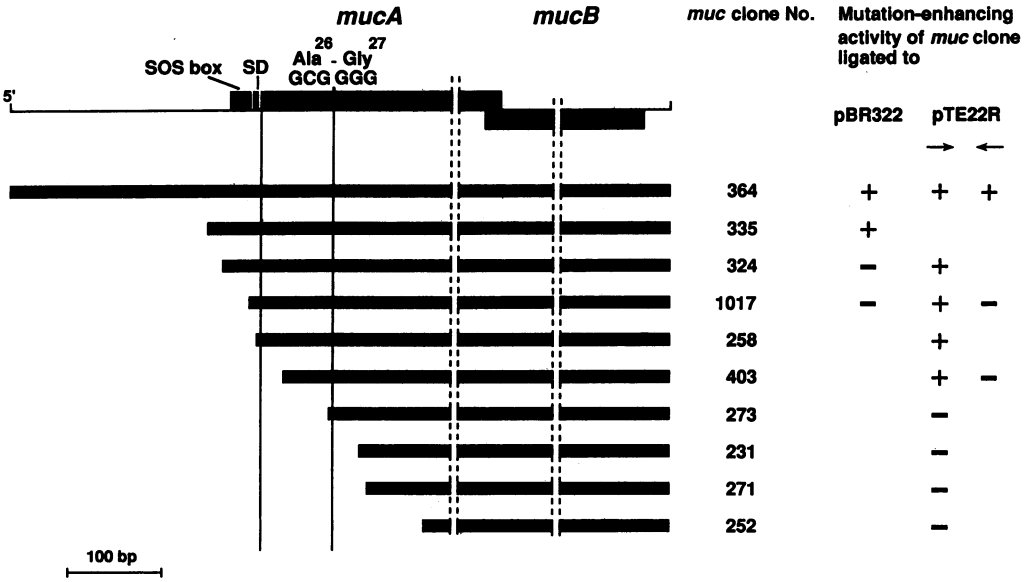

FIG. 3. Mutation-enhancing activities of *mucAB* clones with the 5′ deletions indicated in Fig. 2, examined by the all-or-none test with the noninducible *lexA3* mutant of *E. coli* ZA81 after MMS treatment. Arrows indicate the direction of insertion of *mucAB* clones.

total doses of 1 mg of antigen into female Hartley guinea pigs (Nihon SLC Co., Shizuoka, Japan) initially 4 weeks old. Sera of the animals were tested for reactivity with ^{125}I-labeled antigens and then for reactivity with MucA and MucB protein, respectively, by Western blotting. The useful antibodies for detection of MucA and MucB proteins were those raised to synthetic polypeptides CO19 and CO1B, respectively.

Analysis of MucA and MucB proteins. *E. coli* and *B. subtilis* cells were grown as described previously. To study MucA cleavage, 1 or 5 μg of mitomycin C (MMC; Kyowa Hakko, Tokyo, Japan) per ml, 1/100 diluted MMS, or 1 μg of MNNG per ml was added to cultures in the exponential growth phase for 2 h. Cells were harvested in the late-exponential-growth phase, washed twice with 10 mM Tris–1 mM EDTA (pH 7.4), and concentrated 20-fold in loading buffer containing 1% sodium dodecyl sulfate (SDS). Cells were ruptured by pulsed sonication for 30 s (*E. coli*) or 2 min (*B. subtilis*) in a sonicator (model 200 Sonifier; Branson, Danbury, Conn.). Samples of 20 μg of proteins were subjected to electrophoresis in 4 to 20% SDS–polyacrylamide gel. The separated proteins were transferred to a membrane filter and tested for immunoreactivities to anti-MucA and anti-MucB antibodies by treatment with biotinylated antibody to guinea pig immunoglobulin G (Vector Laboratories, Burlingame, Calif.) and then with H_2O_2.

Nucleotide sequence accession number. The nucleotide sequence for *muc*364 has been assigned DDBJ, EMBL, and GenBank accession no. D90147.

RESULTS

Survival- and mutation-enhancing activities of *mucAB* in *E. coli* and *B. subtilis*. The shuttle vector pTE22R (Fig. 1) was constructed to examine the survival- and mutation-enhancing activities of *mucAB* in *E. coli* and *B. subtilis*. pTE22R confers *E. coli* with resistance to ampicillin (Amp^r) derived from *E. coli* plasmid pBR322 and resistance to chloramphenicol (Cm^r) derived from *B. subtilis* plasmid pE194, but it confers *B. subtilis* with only Cm^r.

By repeated digestion of pKM101 with restriction enzymes and *Bal* 31 exonuclease and successive cloning into pBR322, a 1,978-bp DNA fragment (*muc*364) containing the whole *mucAB* operon was obtained. Figure 2 shows the nucleotide sequence of *muc*364. The *mucB* portion is abbreviated, since it was the same as that determined by Perry et al. (42). However, the *mucA* sequence contained one additional codon, CGG, as the 13th codon, encoding Arg-13 of the MucA protein. The putative RecA cleavage site of MucA is therefore Ala-26–Gly-27, instead of Ala-25–Gly-26. Various deletions of *mucAB* were further produced by digestion of *muc*364 with exonuclease *Bal* 31 from the 5′ end to the region of the sequence encoding Ala-26–Gly-27 in MucA (Fig. 2 and 3), and each DNA fragment was cloned into pBR322 and pTE22R, respectively.

Fig. 4a and b show the effects of the two *mucAB*-containing plasmids, pTE30 (*muc*364-pBR322) and pTE31 (*muc*1017-pTE22R), on the survival and mutation of the noninducible *lexA3* mutant of *E. coli* ZA81 after UV irradiation, together with the effects of *umuDC*. *muc*364 contained the whole *mucAB* operon. The partially synthesized 1,734-bp *muc*1017 contained the SD sequence and the intact coding regions of *mucA* and *mucB* but lacked the SOS box (Fig. 2). By introducing pTE30 (*muc*364), survival of *E. coli* ZA81 was greatly enhanced, indicating repair of UV-induced lethal damage. The survival-enhancing effect of pTE31 (*muc*1017) was less, in spite of abundant production of MucA and MucB proteins by derepressed SOS box-free *mucAB* genes, as shown below. pTA100 (*umuDC*) had a slight but significant effect on survival. Vector alone had no effect. *E. coli* ZA81 was hardly mutable with UV irradiation, and its spontaneous His$^+$ mutation frequency was as low as 10^{-7}. However, by introducing *muc*364, the frequency of UV-induced His$^+$ mutations was greatly increased (13- to 70-fold) in the observed UV dose range. The spontaneous His$^+$ mutation frequency was 1×10^{-6} to 2×10^{-6}. pTE31 (*muc*1017) and pTA100 (*umuDC*) enhanced the mutation frequency to the same extent as pTE30 (*muc*364). These effects of *mucAB* were also reproducibly observed with another noninducible *lexA102* mutant of *E. coli*, CM611 (data not shown). The mutation-enhancing effect of *mucAB* was greater, but the survival-enhancing effect was smaller in *E. coli* cells treated with MMS than in those cells treated with UV irradiation (data not shown).

These effects of *mucAB* were also found with inducible *E. coli* WP2s after UV irradiation, as seen with the original R factor plasmids in *S. typhimurium* (24, 25, 34). No survival- or mutation-enhancing effect of *mucAB* after UV irradiation was seen in the RecA$^-$ mutant of *E. coli* WP100 (*recA56*), as already reported for the original R factor plasmids in Rec$^-$ *S. typhimurium* (25) and RecA$^-$ *E. coli* (32, 54, 55).

The mutation-enhancing activities of *mucAB* with various deletions in the 5′ end of *mucA* were examined with noninducible ZA81 as the host, which gave all-or-none results after treatment with MMS (Fig. 3). Tests on *mucAB* fragments ligated into pBR322 showed that the mutation-enhancing activity was preserved in the deleted fragment provided that the promoter region remained intact but was lost when the deletion extended 6 bp upstream of the SOS box (*muc*324), presumably because the promoter was disrupted. However, this activity could be restored by ligating the deleted *mucAB* fragments to the MLS^r promoter of the vector pTE22R in frame with ATG of the MLS^r gene. The mutation-enhancing activity was finally lost when the deletion in *mucA* extended to the region of the sequence encoding Ala-26–Gly-27.

The alkylating agent-sensitive mutant TKJ6951 of *B. subtilis* carrying a hypersensitive suppressible *his-101* mutation was tested for expression of *mucAB* activities in enhancing cell survival and mutation frequency after mutagenic treatment, by using plasmid pTE31 (*muc*1017). These activities were clearly seen after treatment with MNNG of TKJ6951 containing pTE31 (Fig. 4c and d). The mutation frequency was increased fivefold by pTE31. Treatment with MMS gave a smaller enhancing effect on cell survival (data not shown). Similar but less marked effects were observed with the UV-sensitive mutant TKJ5211 containing pTE31 after treatment with MMS (data not shown).

MucA and MucB proteins. The MucA and MucB proteins produced by *mucAB* in *E. coli* and *B. subtilis* were studied by Western blot analysis by using guinea pig antibodies raised to synthetic polypeptides, i.e., CO19 for MucA and CO1B for MucB. As shown in Fig. 5a, MucA was formed in *E. coli*. The 17-kDa band of MucA was barely detectable in lysates of *E. coli* WP2s containing pTE30 (*muc*364) (Fig. 5a, lane 1) but was much clearer in lysates of cells containing pTE31 (*muc*1017) (lane 3), possibly due to the absence of the SOS box in *muc*1017 and therefore the absence of LexA-mediated repression. Upon treatment with MMC, a second band of MucA appeared at 14 kDa, indicating cleavage of the 17-kDa MucA at the putative cleavage site, Ala-26–Gly-27,

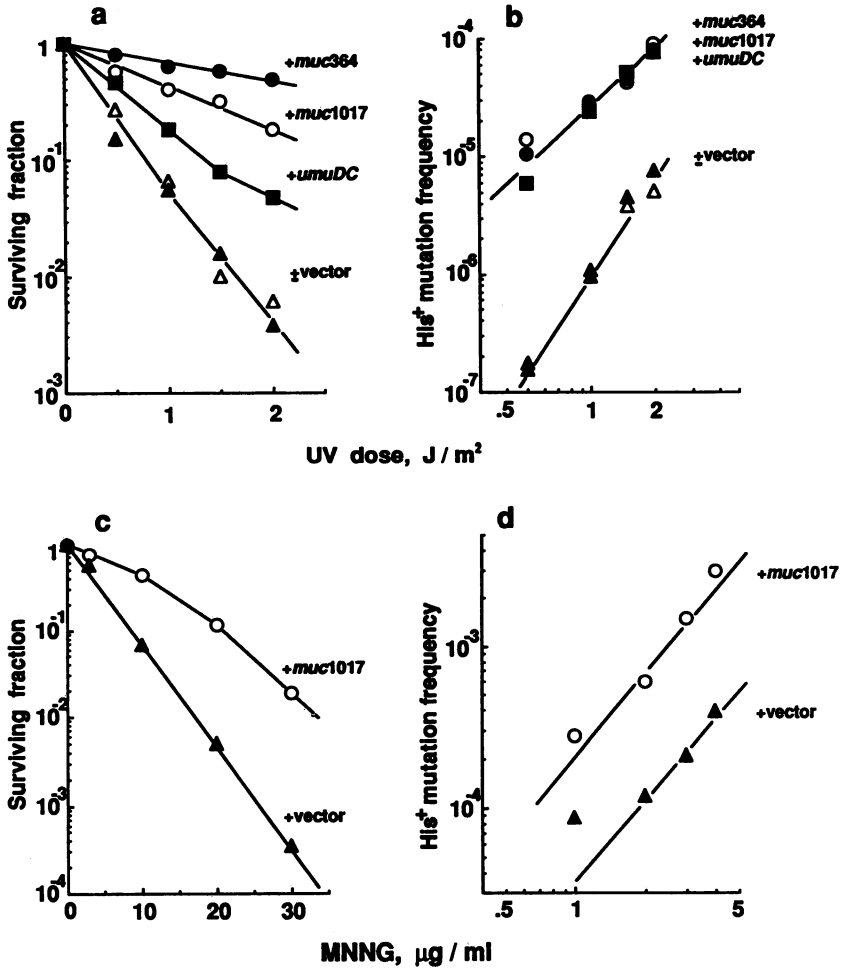

FIG. 4. Effects of *mucAB* on survival and induction of mutation in *E. coli* (a and b) and *B. subtilis* (c and d). The *E. coli* noninducible *lexA3* mutant ZA81 was irradiated with UV light; the *B. subtilis* alkylation-sensitive mutant TKJ6951 was treated with MNNG. Symbols: ●, pTE30 (*muc*364); ○, pTE31 (*muc*1017); ■, pTA100 (*umuDC*); ▲, vector pTE22R; △, without plasmid.

to a 14-kDa fragment (lanes 2 and 4) and an undetectable 3-kDa fragment.

In the noninducible mutants of *E. coli*, ZA81 (*lexA3*) and HB101 (*recA13*), the presence of MucA was again barely detectable with *muc*364 (data not shown). However, with *muc*1017, the formation of MucA was clearly seen both in noninducible LexA$^-$ and in RecA$^-$ mutants of *E. coli* (Fig. 5a, lanes 6 and 11). The cleavage of MucA upon treatment with MMC occurred in the noninducible *lexA3* mutant (lane 7) but not in the RecA$^-$ mutant (lane 12). Spontaneous cleavage of MucA without mutagenic treatment was observed in the *lexA71*(Def) *recA441* mutant of *E. coli* GW2730 (lane 9). Egg white lysozyme (EC 3.2.1.17) reacted with the antiserum and was a useful 14-kDa position marker to identify the cleaved MucA protein (lane 14). The expected formation of fused protein between deleted MucA and the 8.8-kDa portion of the MLS^r protein could not be detected for some technical reason, possibly low reactivity of the antiserum with the fused protein; however, MucB protein was detected (data not shown).

In *B. subtilis*, formation of 17-kDa MucA was clearly recognized in the two strains containing pTE32 (*muc*1017-pHW1) (Fig. 5b). However, cleavage of MucA was observed only when the alkylating agent-sensitive mutant of *B. subtilis* TKJ6951 containing pTE32 was treated with alkylating agents, i.e., MMS (Fig. 5b, lane 6) and, to a lesser extent, MNNG (lane 5). This cleavage was not seen in *recA1* and *recE4* mutants of *B. subtilis* (lanes 10 and 13). pTE31 (*muc*1017-pTE22R), which carried the additional pBR322 portion in the vector, showed no demonstrable MucA products in *B. subtilis*.

MucB proteins were more clearly detectable than MucA proteins in *E. coli* and *B. subtilis*, possibly because of a better antigenic response of the antibody. The formation of the 46-kDa MucB protein by *mucAB* was observed in all strains of *E. coli* and *B. subtilis* examined (Fig. 6). Mutagenic treatment seemed to enhance the formation of MucB protein in *E. coli* WP2s cells containing *muc*364 (Fig. 6, lane 2). MucB showed no change in size upon mutagenic treatment of the *E. coli* and *B. subtilis* cells tested.

DISCUSSION

Mutation is a common phenomenon in various species of living organisms. Thus it would not be surprising to find a common mechanism of mutagenesis in various different organisms. We thought that the mutation-enhancing activity of *mucA* and *mucB* genes might be expressed in various

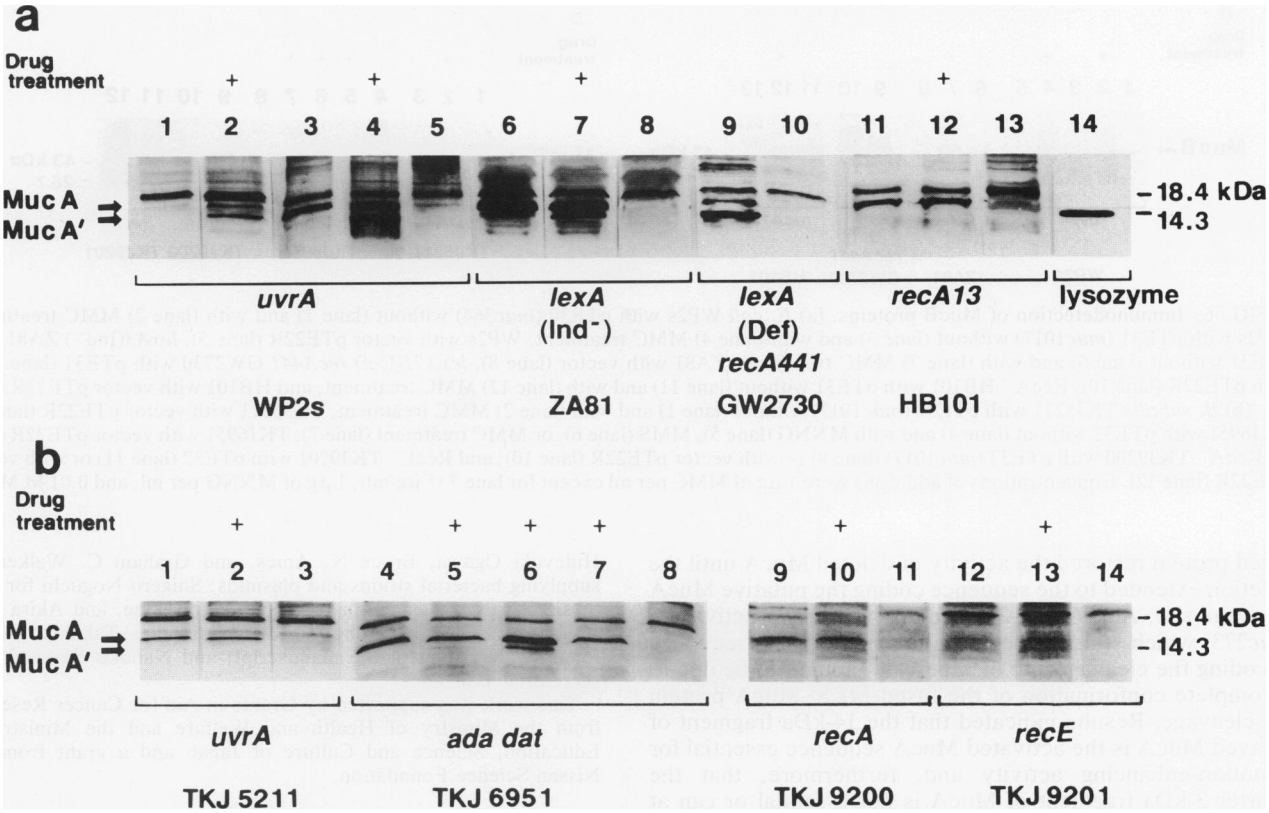

FIG. 5. Immunodetection of MucA proteins. (a) *E. coli* WP2s with pTE30 (*muc*364) without (lane 1) and with (lane 2) MMC treatment, WP2s with pTE31 (*muc*1017) without (lane 3) and with (lane 4) MMC treatment, WP2s with vector pTE22R (lane 5), *lexA3*(Ind⁻) ZA81 with pTE31 without (lane 6) and with (lane 7) MMC treatment, ZA81 with vector pTE22R (lane 8), *lexA71*(Def) *recA441* GW2730 with pTE31 (lane 9), GW2730 with vector pTE22R (lane 10), RecA⁻ HB101 with pTE31 without (lane 11) and with (lane 12) MMC treatment, and HB101 with vector pTE22R (lane 13). Lane 14, egg white lysozyme. MMC concentrations were 1 μg/ml (lanes 7 and 12) or 5 μg/ml (lanes 2 and 4). (b) *B. subtilis* TKJ5211 with pTE32 (*muc*1017) without (lane 1) and with (lane 2) MMC treatment; TKJ5211 with vector pTE22R (lane 3); TKJ6951 with pTE32 without (lane 4) and with treatment with MNNG (lane 5), MMS (lane 6), and MMC (lane 7); TKJ6951 with vector pTE22R (lane 8); RecA⁻ TKJ9200 with pTE31 without (lane 9) and with (lane 10) MMS treatment; TKJ9200 with vector pTE22R (lane 11); RecE⁻ TKJ9201 with pTE32 without (lane 12) and with (lane 13) MMS treatment; and TKJ9201 with vector pTE22R (lane 14). Concentrations of additions were 1 or 5 μg of MMC per ml (lanes 2 and 7); 1 μg of MNNG per ml; and 0.01 M MMS. MucA', 14-kDa fragment of cleaved MucA.

organisms. In this study, we examined the nature of *mucAB* activity in *E. coli* and *B. subtilis*.

Two clones of *mucAB*, *muc*364 and *muc*1017, were used extensively in this study. Clone *muc*364 contained the whole *mucAB* operon, whereas *muc*1017 lacked the SOS box. *muc*364 carried on pTE30 expressed activity only in *E. coli*, possibly because of the species-specific limitation of the promoter activity. When *muc*1017 was ligated downstream to the MLS^r promoter of the *B. subtilis* plasmid in the shuttle vector, the *mucAB* activity was expressed in both *E. coli* and *B. subtilis* cells.

The noninducible *lexA3* mutant of *E. coli* ZA81 became mutable by introducing *mucAB*. The noninducible nature of the *lexA3* mutant is due to the inability of the mutant LexA repressor to be cleaved upon mutagenic treatment and therefore to the absence of the capacity to derepress SOS-regulated genes (17). The effect of *mucAB* on noninducible *E. coli* has been examined with positive (32, 55) and negative (39) results. We think that this discrepancy was due to instability of the large plasmid, because we observed fragmentation of the 35.4-kb pKM101 in the *lexA3* host in parallel with loss of *mucAB* activity (data not shown).

The idea that cleavage of MucA protein mediated by activated RecA influences the survival- and mutation-enhancing activities of *mucAB* has been suggested from studies on UmuD (4, 37, 46). Western blot analysis showed the formation of gene products of *mucA* and *mucB* in *E. coli* strains containing *muc*364 and *muc*1017, i.e., 17-kDa MucA and 46-kDa MucB proteins, and cleavage of MucA into a 14-kDa fragment and an undetectable 3-kDa fragment upon mutagenic treatment of RecA$^+$ *E. coli*. The MucA cleavage occurred in the noninducible *lexA3* mutant of *E. coli*. Specifically, *recA13* was unable to promote cleavage. The spontaneous cleavage seen in the *lexA71*(Def) *recA441* mutant is thought to be due to the repression-deficient nature of this mutant together with leaky expression of the RecA441 protein. Shiba et al. (45) claimed that uncleaved MucA could be active, but we did not examine this point in the present work.

In this study, various sizes of *mucAB* deletions ligated in frame to the MLS^r promoter were prepared. These constructs were expected to produce deleted MucA protein fused with part of the MLS^r protein, containing 41 amino acids encoded by the 123-bp sequence of the MLS^r gene, together with intact MucB protein. Although the fused protein could not be detected, our results indicated that this

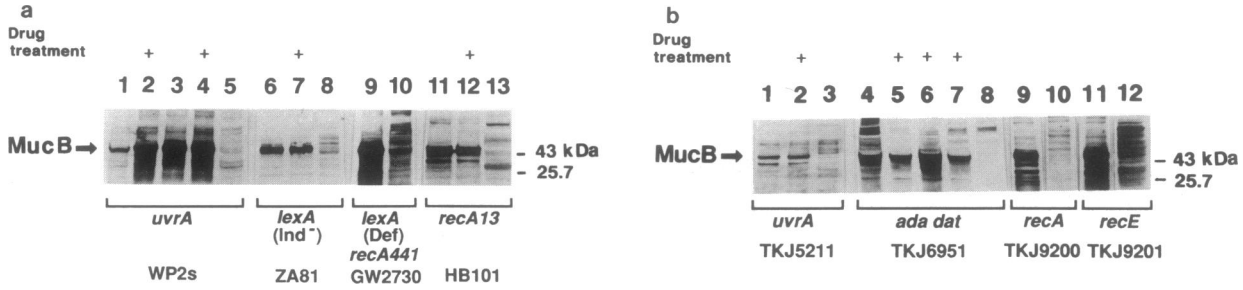

FIG. 6. Immunodetection of MucB proteins. (a) *E. coli* WP2s with pTE30 (*muc*364) without (lane 1) and with (lane 2) MMC treatment, WP2s with pTE31 (*muc*1017) without (lane 3) and with (lane 4) MMC treatment, WP2s with vector pTE22R (lane 5), *lexA3*(Ind$^-$) ZA81 with pTE31 without (lane 6) and with (lane 7) MMC treatment, ZA81 with vector (lane 8), *lexA71*(Def) *recA441* GW2730 with pTE31 (lane 9) or with pTE22R (lane 10), RecA$^-$ HB101 with pTE31 without (lane 11) and with (lane 12) MMC treatment, and HB101 with vector pTE22R (lane 13). (b) *B. subtilis* TKJ5211 with pTE32 (*muc*1017) without (lane 1) and with (lane 2) MMC treatment; TKJ5211 with vector pTE22R (lane 3); TKJ6951 with pTE32 without (lane 4) and with MNNG (lane 5), MMS (lane 6), or MMC treatment (lane 7); TKJ6951 with vector pTE22R (lane 8); RecA$^-$ TKJ9200 with pTE32 (*muc*1017) (lane 9) or with vector pTE22R (lane 10); and RecE$^-$ TKJ9201 with pTE32 (lane 11) or with vector pTE22R (lane 12). Concentrations of additions were 1 μg of MMC per ml except for lane 7 (5 μg/ml), 1 μg of MNNG per ml, and 0.01 M MMS.

fused protein restored the activity of deleted MucA until the deletion extended to the sequence coding the putative MucA cleavage site, Ala-26–Gly-27. The loss of *mucAB* activity in *muc*273, which still retained 6 bp upstream of the sequence encoding the cleavage site of MucA, is thought to be due to incomplete conformation of the fused MLS^r-MucA protein for cleavage. Results indicated that the 14-kDa fragment of cleaved MucA is the activated MucA sequence essential for mutation-enhancing activity and, furthermore, that the shorter 3-kDa fragment of MucA is not essential or can at least be replaced by a foreign protein. When the *mucA* coding region was intact, as in *muc*1017, formation of a 17-kDa MucA protein instead of a 25.8-kDa fused protein was observed in both *E. coli* and *B. subtilis*, indicating that translation started at the intact initiation codon of *mucA* under remote regulation by the MLS^r promotor.

B. subtilis differs in many features from *E. coli*. *B. subtilis* has a more rigid autonomous mechanism for control of DNA replication than *E. coli* (40). In this study, the survival- and mutation-enhancing activities of *mucAB* were found in *B. subtilis* treated with an alkylating agent, coincident with cleavage of MucA. This cleavage did not occur in RecA$^-$ and RecE$^-$ *B. subtilis*. Lovett and Roberts have shown cleavage of the *E. coli* LexA repressor by *B. subtilis* Rec protein in vitro (23), and de Vos and Venema have shown activity of the *B. subtilis* protein similar to that of the *E. coli* protein (7). These results indicated that activity equivalent to the RecA function of *E. coli* exists in *B. subtilis* and, furthermore, that there is a common mutagenic pathway in *E. coli* and *B. subtilis*. The high sensitivity of this cleavage to alkylating agents indicates that alkylating DNA damage is a potent inducer of this RecA-like activity and the SOS response of *B. subtilis*.

The transposonlike structure of *mucAB* shown by Langer et al. (16) indicates the transferable nature of *mucAB* or related genes and their common activity in a wide variety of cells. In an accompanying paper (53), we show an oncogene-like activity of *mucAB* ligated to mouse metal-inducible promoter in mouse BALB 3T3 cells.

ACKNOWLEDGMENTS

We thank Kunitada Shimotohno, Takashi Okamoto, Hiroyasu Esumi, Nobuo Munakata, and Sohei Kondo for useful suggestions and discussions; Sueharu Horinouchi, Minako Nagao, Keiji Wakabayashi, Takeshi Kato, Toshihiro Ohta, Fumiko Moroshoshi, Hideyuki Ogawa, Bruce N. Ames, and Graham C. Walker for supplying bacterial strains and plasmids; Shigeru Noguchi for synthesizing DNA; Ken Yamaguchi, Teruaki Sekine, and Akira Ootsuyama for help in preparing guinea pig antibodies; Takehiko Nohmi for critical reading of the manuscript; and Nahoko Sawasaki for secretarial assistance.

This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare and the Ministry of Education, Science and Culture of Japan and a grant from the Nissan Science Foundation.

REFERENCES

1. **Balganesh, M., and J. K. Setlow.** 1985. Genes from plasmid pKM101 in *Haemophilus influenzae*: separation of functions of *mucA* and *mucB*. Proc. Natl. Acad. Sci. USA **82**:7753–7756.
2. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
3. **Bridges, B. A., R. P. Mottershead, M. A. Rothwell, and M. H. L. Green.** 1972. Repair deficient bacterial strains suitable for mutagenicity screening: tests with the fungicide Captan. Chem.-Biol. Interact. **5**:77–84.
4. **Burckhardt, S. E., R. Woodgate, R. H. Scheuermann, and H. Echols.** 1988. UmuD mutagenesis protein of *Escherichia coli*: overproduction, purification, and cleavage by RecA. Proc. Natl. Acad. Sci. USA **85**:1811–1815.
5. **Chou, P. Y., and G. D. Fasman.** 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. **47**:45–138.
6. **Davis, R. W., D. Botstein, and D. Roth, Jr.** 1980. Advanced bacterial genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **de Vos, W. M., and G. Venema.** 1983. Identification and regulation of the recE gene product. Mol. Gen. Genet. **190**:56–64.
8. **Drabble, W. T., and B. A. D. Stocker.** 1968. R (transmissible drug-resistance) factors in *Salmonella typhimurium*: pattern of transduction by phage P22 and ultraviolet-protection effect. J. Gen. Microbiol. **53**:109–123.
9. **Dubnau, D., and C. Cirigliano.** 1974. Genetic characterization of recombination-deficient mutants of *Bacillus subtilis*. J. Bacteriol. **117**:488–493.
10. **Green, M. H. L., and W. J. Muriel.** 1976. Mutation testing using Trp$^+$ reversion in *Escherichia coli*. Mutat. Res. **38**:3–32.
11. **Horii, T., T. Ogawa, T. Nagatani, T. Hase, H. Matsubara, and H. Ogawa.** 1981. Regulation of SOS functions: purification of *E. coli lexA* protein and determination of its specific site cleaved by the RecA protein. Cell **27**:515–522.
12. **Horinouchi, S., and B. Weisblum.** 1982. Nucleotide sequence and functional map of pE194, a plasmid that specifies inducible

resistance to macrolide, lincosamide, and streptogramin type B antibiotics. J. Bacteriol. **150**:804–814.
13. **Kato, T., and Y. Shinoura.** 1977. Isolation and characterization of mutants of *Escherichia coli* deficient in induction of mutations by ultraviolet light. Mol. Gen. Genet. **156**:121–131.
14. **Kitagawa, Y., E. Akaboshi, H. Shinagawa, T. Horii, H. Ogawa, and T. Kato.** 1985. Structural analysis of the *umu* operon required for inducible mutagenesis in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:4336–4340.
15. **Krueger, J. H., S. J. Elledge, and G. C. Walker.** 1983. Isolation and characterization of Tn*5* insertion mutations in the *lexA* gene of *Escherichia coli*. J. Bacteriol. **153**:1368–1378.
16. **Langer, P. J., W. G. Shanabruch, and G. C. Walker.** 1981. Functional organization of plasmid pKM101. J. Bacteriol. **145**: 1310–1316.
17. **Little, J. W., S. H. Edmiston, L. Z. Pacelli, and D. W. Mount.** 1980. Cleavage of the *Escherichia coli LexA* protein by the *recA* protease. Proc. Natl. Acad. Sci. USA **77**:3225–3229.
18. **Little, J. W., and D. W. Mount.** 1982. The SOS regulatory system of *Escherichia coli*. Cell **29**:11–22.
19. **Little, J. W., D. W. Mount, and C. R. Yanish-Perron.** 1981. Purified *lexA* protein is a repressor of the *recA* and *lexA* genes. Proc. Natl. Acad. Sci. USA **78**:4199–4203.
20. **Lodwick, D., D. Owen, and P. Strike.** 1990. DNA sequence analysis of the *imp* UV protection and mutation operon of the plasmid TP110: identification of a third gene. Nucleic Acids Res. **18**:5045–5050.
21. **Love, P. E., M. J. Lyle, and R. E. Yasbin.** 1985. DNA damage-inducible (*din*) loci are transcriptionally activated in competent *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **82**:6201–6205.
22. **Lovett, C. M., P. E. Love, and R. E. Yasbin.** 1988. SOS-like induction in *Bacillus subtilis*: induction of the RecA protein analog and a damage-inducible operon by DNA damage in Rec$^+$ and DNA repair-deficient strains. J. Bacteriol. **170**:1467–1474.
23. **Lovett, C. M., and J. W. Roberts.** 1985. Purification of a RecA protein analogue from *Bacillus subtilis*. J. Biol. Chem. **260**: 3305–3313.
24. **MacPhee, D. G.** 1972. Effect of an R factor on resistance of *Salmonella typhimurium* to radiation and chemical treatment. Mutat. Res. **14**:450–453.
25. **MacPhee, D. G.** 1973. Effect of *rec* mutations on the ultraviolet-protecting and mutation-enhancing properties of the plasmid R-Utrecht in *Salmonella typhimurium*. Mutat. Res. **19**:357–359.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Marcucci, L., F. Gigliani, P. A. Battaglia, R. Bosi, E. Sopreno, and R. Elli.** 1986. Cellular response to DNA damage is enhanced by the pR plasmid in mouse cells and in *Escherichia coli*. Mol. Cell. Biol. **6**:586–592.
28. **Marsh, L., and G. C. Walker.** 1985. Cold sensitivity induced by overproduction of UmuDC in *E. coli*. J. Bacteriol. **162**:155–161.
29. **Marsh, L., and G. C. Walker.** 1987. New phenotypes associated with *mucAB*: alteration of a *mucA* sequence homologous to the *lexA* cleavage site. J. Bacteriol. **169**:1818–1823.
30. **Maxam, A. M., and W. Gilbert.** 1977. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol. **65**:499–560.
31. **McCann, J., N. E. Spingarn, J. Kobori, and B. N. Ames.** 1975. Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids. Proc. Natl. Acad. Sci. USA **72**:5135–5139.
32. **Monti-Bragadin, C., N. Babudri, and L. S. Samer.** 1976. Expression of the plasmid pKM101-determined DNA repair system in *recA*$^-$ and *lex*$^-$ strains of *Escherichia coli*. Mol. Gen. Genet. **145**:303–306.
33. **Morohoshi, F., and N. Munakata.** 1990. Isolation of a *Bacillus subtilis* mutant defective in constitutive O^6-alkylguanine-DNA alkyltransferase. Mutat. Res. **235**:15–23.
34. **Mortelmans, K. E., and B. A. D. Stocker.** 1976. Ultraviolet light protection, enhancement of ultraviolet mutagenesis, and mutator effect of plasmid R46 in *Salmonella typhimurium*. J. Bacteriol. **128**:271–282.
35. **Mount, D. W., K. B. Low, and S. J. Edmiston.** 1972. Dominant mutations (*lex*) in *Escherichia coli* K-12 which affect radiation sensitivity and frequency of ultraviolet light-induced mutations. J. Bacteriol. **112**:886–893.
36. **Myers, R. M., L. S. Lerman, and T. Maniatis.** 1985. A general method for saturation mutagenesis of cloned DNA fragments. Science **229**:242–247.
37. **Nohmi, T., J. R. Battista, L. A. Dodson, and G. C. Walker.** 1988. RecA-mediated cleavage activates UmuD for mutagenesis: mechanistic relationship between transcriptional derepression and posttranslational activation. Proc. Natl. Acad. Sci. USA **85**:1816–1820.
38. **Nohmi, T., A. Hakura, Y. Nakai, M. Watanabe, S. Murayama, and T. Sofuni.** 1991. *Salmonella typhimurium* has two homologous but different *umuDC* operons: cloning of a second *umuDC*-like operon present in a 60-megadalton cryptic plasmid of *S. typhymurium*. J. Bacteriol. **173**:1051–1063.
39. **Nunoshiba, T., and H. Nishioka.** 1987. Effect of pKM101 plasmid on lethal and mutagenic damage in UV-irradiated *E. coli* strains. Mutat. Res. **183**:39–44.
40. **Ogasawara, N., M. Fujita, S. Moriya, T. Fukuoka, M. Hirano, and H. Yoshikawa.** 1989. Comparative anatomy of *oriC* of eubacteria, p. 287–295. *In* K. Drlica and M. Riley (ed.), The bacterial chromosome. American Society for Microbiology, Washington D.C.
40a. **Ohta, T.** Unpublished results.
41. **Okubo, S., and T. Yanagida.** 1968. Isolation of a suppressor mutant in *Bacillus subtilis*. J. Bacteriol. **95**:1187–1188.
42. **Perry, K. L., S. J. Elledge, B. B. Mitchell, L. Marsh, and G. C. Walker.** 1985. A comparative analysis of the *umuDC* and *mucAB* operons whose products are required for UV and chemical mutagenesis: homology among the UmuD and MucA proteins and LexA repressor. Proc. Natl. Acad. Sci. USA **82**:4331–4335.
43. **Potter, A. A., E. R. Nestman, and V. N. Iyer.** 1984. Introduction of the plasmid pKM101-associated *muc* genes into *Saccharomyces cerevisiae*. Mutat. Res. **131**:197–204.
44. **Radman, M.** 1975. SOS repair hypothesis: phenomenology of an inducible DNA repair which is accompanied by mutagenesis, p. 355–367. *In* P. C. Hanawalt and R. B. Setlow (ed.), Molecular mechanisms for repair of DNA, part A. Plenum Publishing Corp., New York.
45. **Shiba, T., H. Iwasaki, A. Nakata, and H. Shinagawa.** 1990. Proteolytic processing of MucA protein in SOS mutagenesis: both processed and unprocessed MucA may be active in the mutagenesis. Mol. Gen. Genet. **224**:169–176.
46. **Shinagawa, H., H. Iwasaki, T. Kato, and A. Nakata.** 1988. RecA* protein-dependent cleavage of UmuD protein upon SOS mutagenesis. Proc. Natl. Acad. Sci. USA **85**:1806–1810.
47. **Slilaty, S. N., and J. W. Little.** 1987. Lysine-156 and serine-119 are required for *lexA* repressor cleavage: a possible mechanism. Proc. Natl. Acad. Sci. USA **84**:3987–3991.
48. **Smith, C. M., W. H. Koch, S. B. Franklin, P. L. Foster, T. A. Cebula, and E. Eisenstadt.** 1991. Sequence analysis and mapping of the *Salmonella* typhimurium LT2 *umuDC* operon. J. Bacteriol. **172**:4964–4978.
49. **Spikes, D., and J. K. Setlow.** 1989. A plasmid carrying *mucA* and *mucB* genes from pKM101 in *Haemophilus influenzae* and *Escherichia coli*. J. Bacteriol. **171**:5753–5755.
50. **Spizizen, J.** 1958. Transformation of biochemically deficient strains of *Bacillus subtilis* by deoxyribonucleate. Proc. Natl. Acad. Sci. USA **44**:1072–1078.
51. **Tanooka, H.** 1977. Development and applications of *Bacillus subtilis* test systems for mutagens involving DNA-repair deficiency and suppressible auxotrophic mutations. Mutat. Res. **42**:19–32.
52. **Thomas, S. M., H. M. Crowne, S. C. Pidsley, and S. G. Sedgwick.** 1990. Structural characterization of the *Salmonella typhimurium* LT2 *umu* operon. J. Bacteriol. **172**:4979–4987.
53. **Tosu, M., and H. Tanooka.** 1990. Transformation of mouse BALB 3T3 cells by enterobacterial plasmid misrepair gene *mucAB*. Mol. Cell. Biol. **10**:5359–5364.
54. **Upton, C., and R. J. Pinny.** 1983. Expression of eight unrelated

Muc$^+$ plasmids in eleven DNA repair-deficient *E. coli* strains. Mutat. Res. **112**:261–273.
55. **Waleh, N. S., and B. A. D. Stocker.** 1979. Effect of host *lex*, *recA*, *recF*, and *uvrD* genotypes on the ultraviolet light-protecting and related properties of plasmid R46 in *Escherichia coli*. J. Bacteriol. **137**:830–838.
56. **Walker, G. C.** 1984. Mutagenesis and inducible responses of deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48**:60–93.
57. **Watanabe, T.** 1963. Infective heredity of multiple drug resistance in bacteria. Bacteriol. Rev. **27**:87–115.
58. **Witkin, E. M.** 1976. Ultraviolet mutagenesis and inducible DNA repair in *Escherichia coli*. Bacteriol. Rev. **40**:869–907.
59. **Witkin, E. M., and D. L. George.** 1973. Ultraviolet mutagenesis in *polA* and *uvrA* derivatives of *E. coli* B/r: evidence for an inducible error-prone repair system. Genetics **73**:91–108.
60. **Woodgate, R., M. Rajagopalan, C. Lu, and H. Echols.** 1989. UmuC mutagenesis protein of *Escherichia coli*: purification and interaction with UmuD and UmuD'. Proc. Natl. Acad. Sci. USA **86**:7301–7305.