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Mutation and Killing of *Escherichia coli* Expressing a Cloned *Bacillus subtilis* Gene Whose Product Alters DNA Conformation

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Expression of the *Bacillus subtilis* gene coding for SspC, a small, acid-soluble protein, caused both killing and mutation in a number of *Escherichia coli* B and K-12 strains. SspC was previously shown to bind *E. coli* DNA in vivo, and in vitro this protein binds DNA and converts it into an A-like conformation. Analysis of revertants of nonsense mutations showed that SspC caused single-base changes, and a greater proportion of these were at A-T base pairs. Mutation in the *recA* gene abolished the induction of mutations upon synthesis of SspC, but the killing was only slightly greater than in RecA$^+$ cells. Mutations in the *umuC* and *umuD* genes eliminated most of the mutagenic effect of SspC but not the killing, while the *lexA* mutation increased mutagenesis but did not appreciably affect the killing. Since there was neither killing nor mutation of *E. coli* after synthesis of a mutant SspC which does not bind DNA, it appears likely that the binding of wild-type SspC to DNA, with the attendant conformational change, was responsible for the killing and mutation. A strain containing the *B. subtilis* gene that is constitutive for the RecA protein at 42°C showed a lower frequency of mutation when that temperature was used to induce the RecA protein than when the temperature was 30°C, where the RecA level is low, suggesting that at the elevated temperature the high RecA level could be inhibiting binding of the *B. subtilis* protein to DNA.

In *Haemophilus influenzae*, a DNA gyrase mutation that resulted in increased DNA supercoiling increased the frequency of spontaneous mutation, while a gyrase mutation that markedly decreased DNA supercoiling reduced the spontaneous mutation frequency below the wild-type level (18). These results suggested that DNA configuration is an important parameter in determining the frequency of spontaneous mutation. In order further to test the latter concept, we decided to analyze the effect on mutation frequency in *Escherichia coli* of one of a group of *Bacillus subtilis* proteins which are known to alter DNA conformation (12). The specific *B. subtilis* protein tested was the product of the *sspC* gene (termed SspC), which is one of the α/β-type small, acid-soluble proteins (SASP) found at high levels in dormant spores (18a). Previous work has shown that SspC (as well as all other α/β-type SASP tested) binds double-stranded DNA in vitro and causes the DNA to undergo a B→A conformational change (12). When SspC or other α/β-type SASP are synthesized in *E. coli*, these proteins reach levels sufficient to saturate up to 35% of the cell's DNA and become associated with the cell's nucleoid, presumably causing a conformational change in the DNA in vivo (17). SspC has also been shown to inhibit transcription of some genes, both in vivo and in vitro (17a).

The present work is an investigation of the mutational effects of SspC synthesis in *E. coli* strains, some of which contain mutations in *E. coli* genes which might be expected to affect such mutagenesis.

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MATERIALS AND METHODS

Bacteria and plasmids. Tables 1 and 2 list the *E. coli* strains and the plasmids and T4 bacteriophage used. Plasmid pPS708^{wt} carries the gene coding for wild-type SspC under control of the promoter of the *lac* operon; consequently, SspC synthesis is inducible by isopropyl-β-D-thiogalactopyranoside (IPTG) (13). Plasmid pPS708^{Ala} encodes a mutant SspC in which a glycine residue conserved in all α/β-type SASP has been converted to an alanine. Whereas SspC^{Ala} is synthesized in *E. coli* at levels identical to those of wild-type SspC, SspC^{Ala} does not bind to DNA either in vivo or in vitro (22).

Bacteria were routinely grown at 37°C in PY medium (20 g of peptone, 10 g of yeast extract, and 5 g of NaCl per liter; adjusted to pH 7 with NaOH). For PY plates, 15 g of agar were added per liter. *E. coli* strains were transformed with plasmids by the $CaCl_2$ method (16), usually from crude preparations of plasmids. The selection for the first five plasmids in Table 2 was with ampicillin (50 μg/ml); the other two plasmids were selected for with chloramphenicol (10 μg/ml). To construct plasmid pET-3SspC, the 0.6-kb *Hin*dIII fragment encompassing the *sspC* structural gene was isolated from pPS708^{wt}. The ends of the latter fragment were filled in with the large fragment of *E. coli* DNA polymerase, and it was then ligated into the *Bam*HI site of plasmid pET-3 after the *Bam*HI ends had also been filled in. The ligation mixture was then used to transform strain HMS174 to ampicillin resistance, and colonies carrying the *sspC* gene were detected by colony hybridization. A strain with a plasmid in which the *sspC* coding sequence was just downstream of and in the correct orientation relative to the plasmid's T7 RNA polymerase promoter was then identified by digestion with *Eco*RV (13). The resulting plasmid, pET-3SspC, was then introduced into *E. coli* BL21 and BL21(DE3), with or without the last two plasmids listed in Table 2.

Mutation assays. Cells were grown at 37°C (unless other-

TABLE 1. Strains of *E. coli* used

Strain	Relevant genotype	Reference	Source
HB101	*leu thi pro recA13*	2	Alan Rosenberg
RR1	Like HB101, but RecA$^+$	1	Michael Holland
AB1157	*his arg thr thi leu pro*	7	J. Eugene LeClerc
AB2463	Like AB1157, but *recA13*	8	Barbara Bachmann
GW3198	Like AB1157, but *umuC36*	4	Graham Walker
GW3200	Like AB1157, but *umuD44*	4	Graham Walker
JL2491	Like AB1157, but *lexA300*(del) *sulA*::Tn*5*	15a	Ken Roland
GC3217	Like AB1157, but *recA441 sfi ilvts*	6	Evelyn Witkin
BL21	B strain	21	Alan Rosenberg
BL21(DE3)	Like BL21, but *lac* promoter and gene for T7 RNA polymerase	21	Alan Rosenberg
WU3610	B/r derivative, *tyr leu*	5, 25	Rosalie Elespuru
WU3610 (Su1)	Like WU3610, but Su1	3	Rosalie Elespuru
WU3610 (SuB)	Like WU3610, but SuB	3	Rosalie Elespuru
HMS174	K-12 derivative, *recA1*	21	Alan Rosenberg

wise noted) to an optical density at 675 nm of 0.4, incubated for 2.25 h with or without 0.5 mM IPTG, and finally centrifuged and resuspended in cold 0.15 M NaCl, usually at a final concentration of around 3×10^8/ml. The number of viable cells was measured after an appropriate dilution by plating them on PY agar. Unless stated otherwise, mutation to prototrophy was assayed by plating 0.1 ml from the saline suspension on minimal E medium (23) containing all but one of the required supplements indicated in Table 1. WU3610 (5, 25) was assayed for mutation on SEM medium (25). Colonies on PY medium were counted after 1 day of incubation. Cells on minimal supplemented medium were counted after 3 days.

In contrast to some previous observations (28a), no "jackpot" mutations were seen from the platings of liquid cultures used for IPTG treatment and controls, and thus there was reasonable agreement between the different determinations. Where there were a number of different determinations, cultures were started from different colony isolates and small inocula and then grown to the optical density noted above without additional subculture. A typical set of four separate determinations in which there were few mutations is as follows: a total of eight mutant colonies (on 10 plates), none (on 9 plates), two (on 9 plates), and two (on 14 plates).

As found previously by others (3a, 28a), the apparent mutation frequency of strain WU3610 lacking the plasmid increased with decreasing numbers of viable cells on the plate (Table 3). The reason for this phenomenon is that the SEM minimal medium used for WU3610 (26) contains a small amount of nutrient broth and thus allows the limited growth of nonmutants. The number of replications of these cells increases with decreasing number of cells plated, with the result that the number of cells on the plates eventually becomes independent of the number initially placed there. Consequently, many of the mutants on these plates have arisen during the growth on the plates, so that the mutation frequency on the basis of the total number of cells after this growth, which is a constant, is also a constant. However, the mutation frequency of IPTG-treated cells containing plasmid pPS708^{wt} was independent of the number of viable cells (Table 3), indicating that the mutations had arisen during the 2.25-h incubation before plating, and did not markedly increase on the plates. Nevertheless, because of these phenomena, an attempt was made to keep the number of viable cells per plate constant (around 3×10^7) in all determinations of mutation frequency. Since there was a large variation in the killing of the different strains by IPTG induction (Table 4), some strains had to be concentrated after treatment in order to match the number of viable cells of the strain not treated with IPTG or of the strain lacking the plasmid.

TABLE 2. Plasmids and T4 phage used

Plasmid or phage	Relevant properties	Reference	Source
Plasmids			
pDG148	Vector used for construction of pPS708^{wt} and pPS708^{Ala}	20	Patrick Stragier
pPS708^{wt}	Codes for wild-type *B. subtilis* SspC, and SspC is induced by IPTG	13	
pPS708^{Ala}	Like pPS708^{wt}, except SspC^{Ala} does not bind to DNA	22	
pET-3	Cloning vector for genes under control of T7 promoter	21	Alan Rosenberg
pET-3SspC	Gene for *B. subtilis* SspC in pET-3		This work
pLysS	Produces low lysozyme levels	21	Alan Rosenberg
pLysE	Produces high lysozyme levels	21	Alan Rosenberg
T4 phage			
Wild type		10	Rosalie Elespuru
B17		3	Rosalie Elespuru
NG19		3	Rosalie Elespuru
och427		3	Rosalie Elespuru
ps292		3	Rosalie Elespuru
ps205		3	Rosalie Elespuru
B22		3	Rosalie Elespuru

TABLE 3. Variation of apparent mutation frequency of *E. coli* WU3610[a]

Strain	Viable cells/plate	*tyr* to *tyr*$^+$		*leu* to *leu*$^+$	
		Mutant colonies/plate[b]	Frequency of mutation	Mutant colonies/plate[b]	Frequency of mutation
WU3610 (+ and − IPTG)[c]	2.3×10^8	8 (5)	4×10^{-8}	8 (5)	3×10^{-8}
	2.1×10^8	8 (4)	4×10^{-8}	7 (5)	3×10^{-8}
	6.0×10^7	8 (4)	1×10^{-7}	6 (5)	9×10^{-8}
	7.3×10^6	9 (5)	1×10^{-6}	8 (5)	1×10^{-6}
WU3610/pPS708^{wt} (+ IPTG)	5.7×10^7	131 (2)	2.3×10^{-6}	130 (2)	2.3×10^{-6}
	5.6×10^6	16 (8)	2.9×10^{-6}	13 (6)	2.3×10^{-6}

[a] Strains were grown and incubated with or without IPTG, and viable cells and mutants were determined as described in Materials and Methods.
[b] Values in parentheses are the numbers of plates analyzed in this experiment.
[c] Since little or no difference was observed in the effect of IPTG on the total number of viable cells or in the level of mutation without pPS708^{wt}, the mutation data for WU3610 with and without IPTG were added together.

Determination of the nature of SspC-induced mutations. In order to determine the type of mutations induced by SspC, we analyzed the ability of a number of bacteriophage T4 mutants to grow on the *E. coli* mutants (3, 10, 14). The *E. coli* strain used for this analysis was WU3610, a derivative of strain B/r containing an ochre mutation rendering the strain tyrosine dependent and an amber mutation causing leucine dependence. Cells were incubated with or without IPTG treatment as described above, and, after an appropriate dilution in 0.15 M NaCl, they were plated on minimal SEM plates (23, 26) containing either tyrosine or leucine (20 μg/ml) and on PY plates to determine the total number of viable cells. Revertant colonies on the SEM plates were picked, grown to saturation in PY liquid medium, and frozen for subsequent assays of phage growth. Wild-type T4 and phage mutants B17 and NG19 were grown in the Sul suppressor strain, and och427, ps292, ps205, and B22 were grown in SuB. Stocks of these phage were spotted with pipettes on a freshly poured bacterial lawn (10 to 10^2 phages per spot) of each WU3610 *leu*$^+$ or *tyr*$^+$ revertant in T4 top agar as described previously (16), except that NZ amine (enzymatic digest of casein) was substituted for tryptone. Phage growth or lack of it was readily observed after an overnight incubation at 37°C. As noted previously (3), reversion of the *leu* or *tyr* mutations to prototrophy could be due to changes in the stop codon or to a change in a tRNA's anticodon that produced a suppressor tRNA. The specific base change in the latter group of mutations can then be determined by the pattern of growth of the mutant T4 bacteriophage on the *leu*$^+$ or *tyr*$^+$ revertants. A disadvantage to this method is that only the wild-type phage may grow on some of the revertants, indicating that the mutational base change was in the amber or ochre stop codon. However, there is still base sequence information to be gained from the "wild type only" results. For example, with the amber stop codon (TAG), a change of G to A yields another stop codon. While the change of G to either C or T will generate a tyrosine codon, it appears that the insertion of tyrosine at this position may not yield a wild-type protein (3). Consequently, changes in a TAG codon causing reversion of the WU3610 amber mutation must be in an A-T base pair, giving CAG (Gln), GAG (Glu), TCG (Ser), TTG (Leu), AAG (Lys), or TGG (Trp) (3). Obviously, for the ochre stop codon (TAA), reversion of this mutation must involve an alteration of an A-T base pair. A huge advantage in the use of this method over DNA sequence analysis for determination of mutational specificity is that many revertants can be analyzed in a very short time. Thus, one of us (J.K.S.) generated all of the data on 346 revertants in less than 2 months.

TABLE 4. Effect of IPTG treatment on cell viability

Strain	Viability (%)[a]	No. of determinations
AB1157	97	5
AB1157/pPS708^{wt}	8	5
GW3198	107	1
GW3198/pPS708^{wt}	4	3
GW3200	95	1
GW3200/pPS708^{wt}	4	4
GC3217	90	3
GC3217/pPS708^{wt}	0.5	6
GC3217/pPS708^{Ala}	88	1
AB2463/pPS708^{wt}	3	4
JL2491/pPS708^{wt}	6	4
RR1/pPS708^{wt}	12	5
RR1/pDG148	78	2
RR1/pPS708^{Ala}	100	3
HB101/pPS708^{wt}	2	5
BL21/pET-3SspC	100	1
BL21(DE3)/pET-3SspC	0.0009	1
BL21(DE3)/pLysS/pET-3SspC	0.0012	1
BL21(DE3)/pLysE/pET-3SspC	0.03	4
WU3610	100	1
WU3610/pPS708^{wt}	7	4

[a] (No. IPTG treated/no. untreated) × 100.

RESULTS

Killing by IPTG induction. Not surprisingly, strains lacking plasmids encoding SspC were not killed by IPTG induction (Table 4). Similarly, strain BL21/pET-3SspC, which carries no T7 RNA polymerase gene, was also immune to IPTG-induced killing, since the T7 RNA polymerase is required to transcribe genes inserted in the pET-3 vector (21). In contrast, strains in which wild-type SspC synthesis is induced by IPTG exhibited significant killing by IPTG treatment (Table 4). The most sensitive strain was BL21(DE3)/pET-3SspC, which also made the largest amount of SspC after induction (17a). When this strain also contained pLysS, it made a small amount of T7 lysozyme, a substance which inhibits T7 RNA polymerase (21), and was slightly less sensitive to IPTG. The pLysE plasmid causes the production of a large amount of T7 lysozyme (21), and thus the strain with this plasmid and pPS708^{wt} was more than an order of magnitude more resistant to killing by IPTG induction than

TABLE 5. Mutation caused by induction of wild-type SspC synthesis by IPTG[a]

Strain	Plasmid	Presence of IPTG	Reversion to prototrophy	Mutant colonies/plate[b]	Mutation frequency
AB1157	None	+ and −[c]	*leu*$^+$	0.5 (12)	5×10^{-9}
	pPS708^{wt}	+	*leu*$^+$	21 (3)	2×10^{-7}
	pPS708^{wt}	−	*leu*$^+$	0.14 (14)	2×10^{-9}
GC3217[d]	None	+ and −	*leu*$^+$	0.04 (24)	2.4×10^{-9}
	pPS708^{wt}	+	*leu*$^+$	5 (33)	2.2×10^{-7}
	pPS708^{wt}	−	*leu*$^+$	0.09 (34)	4×10^{-9}
	pPS708^{Ala}	+ and −	*leu*$^+$	0.06 (32)	3×10^{-9}
	None	+ and −	*thi*$^+$	0 (16)	$<4 \times 10^{-9}$
	pPS708^{wt}	+	*thi*$^+$	2.1 (8)	1×10^{-7}
	pPS708^{wt}	−	*thi*$^+$	0 (8)	$<1 \times 10^{-9}$
	pPS708^{Ala}	+ and −	*thi*$^+$	0.06 (32)	3×10^{-9}
RR1	pPS708^{wt}	+	*leu*$^+$	36 (5)	2×10^{-6}
	pPS708^{Ala}	+ and −	*leu*$^+$	0 (16)	$<1 \times 10^{-9}$
WU3610	None	+ and −	*tyr*$^+$	8 (4)	1.4×10^{-7}
	None	+ and −	*leu*$^+$	6 (5)	1.0×10^{-7}
	pPS708^{wt}	+	*tyr*$^+$	131 (2)	2.3×10^{-6}
	pPS708^{wt}	+	*leu*$^+$	130 (2)	2.3×10^{-6}
	pPS708^{wt}	−	*tyr*$^+$	19 (10)	4×10^{-7}
	pPS708^{wt}	−	*leu*$^+$	14 (9)	3×10^{-7}

[a] Strains were grown and treated with or without IPTG, and mutation frequencies were determined as described in Materials and Methods. In these experiments, for each strain, the numbers of viable cells plated for mutation frequency determinations were similar ($\sim 3 \times 10^7$).
[b] Values in parentheses are the numbers of plates analyzed.
[c] Since little or no difference was observed in the effect of IPTG on the level of mutation in cells without pPS708^{wt}, the data for IPTG treatment and for no treatment were added together.
[d] The temperature used for GC3217 was 37°C.

the strain containing pLysS. These cells also made considerably less SspC than cells of the other two strains (17a). These data indicate that the killing caused by IPTG resulted from the transcription and translation of the *sspC* gene. In addition, we found that IPTG induction of plasmid pPS708^{Ala} produced no killing (Table 4). Since the product of this mutant *sspC* gene, SspC^{Ala}, does not bind to DNA in vivo or in vitro (22), the data further suggest that killing by wild-type SspC requires binding of the latter to DNA. Although it is clear that wild-type SspC can kill *E. coli*, the mechanism of this killing is not clear. The optical density of cultures treated with IPTG was often not significantly lower than for untreated cultures. Consequently, the loss in viability could not be attributed to cessation of growth or lysis. Almost all strains in which there was significant killing after SspC synthesis were seen by microscopic examination to be filamented.

Mutation caused by SspC synthesis. Analysis of the mutation frequencies for a number of strains with and without IPTG treatment showed that those carrying pPS708^{wt} had a considerable increase in mutation frequency by IPTG induction (Table 5). In contrast, induction of SspC^{Ala} synthesis had no effect on mutation frequency, in accord with the lack of killing by this protein (Tables 4 and 5).

Analysis of the type of mutation causing the reversion to prototrophy in strains with two different nonsense mutations showed that in a strain without pPS708^{wt} about half of the reversion mutations were in the stop codon, with the remainder in various tRNA genes generating suppressor tRNAs (Table 6). Approximately one-third of these mutations were in G-C base pairs. In contrast, 75% of the mutations induced by SspC were in the original nonsense codon, with only 18% at G-C base pairs (Table 6). The data further suggest that all or almost all of the mutations induced by SspC are single-base changes. Eggertsson and Söll (2a) have asserted that suppressors resulting from two or three base changes are usually so rare as to be unobservable. Further, they state that the probability that there are other uncharacterized suppressors involving single-base changes in tRNA is extremely small.

Effect of other genes on SspC-induced mutation and killing. Four *E. coli* genes are known to be important in mutagenesis caused by such factors as UV irradiation: *recA*, *umuC*, *umuD*, and *lexA* (24, 28). The RecA gene product seemed not to be significantly involved in the repair of possible DNA damage induced by SspC, since survival of two otherwise isogenic *recA*$^+$-*recA* pairs was similar after IPTG induction (AB1157/pPS708^{wt}-AB2463/pPS708^{wt} and RR1/pPS708^{wt}-

TABLE 6. DNA base changes in revertants of WU3610 and WU3610/pPS708^{wt} [a]

Presence of pPS708^{wt}	No. of *leu*$^+$ revertants			No. of *tyr*$^+$ revertants		
	GC to TA[b] (Su1)	GC to AT[b] (Su2)	AT changed[c]	GC to AT[b] (SuB)	TA to AT[b] (Su5)	AT changed[c]
−	2	28	31	16	19	37
+	3	23	71	12	15	89

[a] Strains were grown and incubated with IPTG, and revertants were isolated and tested for the base change causing the reversion with wild-type and mutant T4 as described in Materials and Methods. Numbers of revertants are shown for each category.
[b] These mutations were in tRNA genes yielding particular suppressor tRNAs (shown in parentheses).
[c] These mutations were in the nonsense codon of the original mutation causing a *leu* or *tyr* phenotype, since only wild-type phage grew on them.

TABLE 7. Mutation caused by SspC in *E. coli* requires the RecA protein[a]

Strain	Reversion to prototrophy	Presence of plasmid pPS708^{wt}	Presence of IPTG	Mutant colonies/plate[b]	Frequency of mutation
AB2463 (*recA13*)	*leu*$^+$	−	+ and −[c]	0.6 (48)	2×10^{-8}
	leu$^+$	+	+	0.3 (42)	1×10^{-8}
	leu$^+$	+	−	0.6 (26)	2×10^{-8}
	thi$^+$	−	+ and −	0 (32)	$<1 \times 10^{-9}$
	thi$^+$	+	+	0.2 (17)	6×10^{-9}
	thi$^+$	+	−	0 (16)	$<2 \times 10^{-9}$
AB1157 (RecA$^+$) + plasmid from AB2463	*leu*$^+$	+	+	18 (8)	6×10^{-7}
	leu$^+$	+	−	1 (8)	4×10^{-8}
HB101 (*recA13*)	*leu*$^+$	+	+	0 (17)	$<4 \times 10^{-9}$
	leu$^+$	+	−	0 (16)	$<4 \times 10^{-9}$
GC3217 (*recA441*) + plasmid from AB2463	*leu*$^+$	+	+	4 (16)	1.4×10^{-7}
	leu$^+$	+	−	0 (17)	$<2 \times 10^{-9}$

[a] Strains were grown and incubated with or without IPTG, and mutation frequencies were determined as described in Materials and Methods.
[b] Values in parentheses are the numbers of plates analyzed.
[c] The mutation data were added when no plasmid was present, since no difference was observed between IPTG-treated and untreated cells.

HB101/pPS708^{wt}; Table 4). These data contrast with those obtained with UV irradiation, in which the *recA13* mutation in strain AB2463 reduced survival more than 3 orders of magnitude, compared with the survival of the *recA*$^+$ parent, AB1157, down only to about 10% (8).

While the RecA protein appeared not to be significantly involved in SspC cell killing, the RecA protein did appear to affect SspC-induced mutagenesis, because there was little or no mutation caused by the IPTG induction of pPS708^{wt} in *recA* strains (Table 7). In order to make sure that the lack of effect in the *recA* strains was the result of the *recA13* mutation rather than a change in the plasmid, cleared lysates from these strains were made and AB1157 and GC3217, strains normally showing enhanced mutation by IPTG induction, were transformed with plasmids from the *recA* cells. Since the transformants' mutation levels were elevated when the *sspC* gene was turned on (Table 7), it was concluded that the plasmid which had been in the Rec$^-$ strains was functional and, therefore, that the RecA protein was required for SspC-induced mutagenesis.

Previously, it was shown that spontaneous mutation increases in strain GC3217 (*recA441*) when the temperature is shifted to 41°C (6) or 42°C (29) and adenine is present. This effect has been attributed to activation of the RecA protein and induction of its synthesis (24). As noted previously (29), there was some increased mutation in strain GC3217 lacking pPS708^{wt} at 42°C compared with that at 30°C (Table 8). However, the mutations induced by SspC synthesis were less frequent in this strain at 42°C than at 30°C (Table 8). Control experiments showed that the mutation frequency in strain AB1157 by SspC synthesis was similar at 30 and 42°C (Table 8). Previous work has also shown that IPTG induction of pPS708^{wt} in two other RecA$^+$ strains generates similar SspC levels at these two temperatures (17).

As noted above for the *recA13* mutation, mutations in either *umuC* or *umuD* had very little effect on the IPTG-induced killing of strains carrying pPS708^{wt} (Table 4). Just as the *recA13* strains were not mutagenized by SspC synthesis, the *umuC* and *umuD* mutations almost eliminated this mutagenesis (Table 9). However, pPS708^{wt} plasmids from *umuC* and *umuD* strains were active in mutating other strains (Table 9), indicating that the decreased mutation in the *umuC* and *umuD* mutants was not a change in the plasmid.

In contrast to the effect of mutations in *recA*, *umuC*, and *umuD* genes on SspC-induced mutagenesis in *E. coli*, a *lexA* mutation resulted in a greater frequency of mutation after SspC synthesis than occurred with its *lexA*$^+$ parent strain, AB1157 (Tables 5 and 10), although killing was not appreciably different (Table 4).

TABLE 8. Effect of temperature and adenine on SspC-induced mutation during the first hour of IPTG treatment[a]

Strain	Presence of plasmid	Temp (°C) in 1st h of incubation	Presence of IPTG	Mutant colonies/plate[b]	Frequency of mutation
GC3217	+	42	+	14 (16)	5.4×10^{-7}
	+	42	−	0.12 (16)	1×10^{-8}
	+	30	+	129 (16)	1.6×10^{-5}
	+	30	−	0.19 (16)	1.5×10^{-8}
	−	42	+ and −[c]	0.22 (40)	1.8×10^{-8}
	−	30	+ and −[c]	0.05 (39)	5×10^{-9}
AB1157	+	42	+	22 (8)	6×10^{-7}
	+	42	−	0.9 (8)	5×10^{-8}
	+	30	+	31 (8)	5×10^{-7}

[a] Growth before IPTG treatment was at 30°C. Further incubation with or without IPTG and with adenine (100 μg/ml) was for 1 h at 30 or 42°C, followed by the last 1.25 h at 30°C. Plates were incubated at 30°C, and *leu*$^+$ revertants were scored. The total number of viable cells recovered was independent of the temperature during the first hour with IPTG.
[b] Values in parentheses are the numbers of plates analyzed.
[c] Data for IPTG treatment and for no treatment were combined for strains lacking pPS708^{wt}.

TABLE 9. The *umuc36* and *umuD44* mutations prevent most of the SspC-induced mutation in *E. coli*[a]

Strain	Presence of pPS708^{wt}	Presence of IPTG	Reversion to prototrophy	Mutant colonies/ plate[b]	Frequency of mutation
GW3198 (*umuC36*)	−	+ and −[c]	*leu*$^+$	0 (16)	$<2 \times 10^{-9}$
	+	+	*leu*$^+$	0.38 (21)	3×10^{-8}
	+	−	*leu*$^+$	0.14 (21)	5×10^{-9}
GW3200 (*umuD44*)	−	+ and −	*leu*$^+$	0 (16)	$<2 \times 10^{-9}$
	+	+	*leu*$^+$	0.08 (12)	3×10^{-9}
	+	−	*leu*$^+$	0 (12)	$<3 \times 10^{-9}$
GW3198	−	+ and −	*thi*$^+$	0 (16)	$<4 \times 10^{-9}$
	+	+	*thi*$^+$	0.06 (17)	8×10^{-9}
	+	−	*thi*$^+$	0 (17)	$<2 \times 10^{-9}$
GW3200	−	+ and −	*thi*$^+$	0 (16)	$<4 \times 10^{-9}$
	+	+	*thi*$^+$	0.12 (16)	1×10^{-8}
	+	−	*thi*$^+$	0 (16)	$<6 \times 10^{-9}$
GC3217 + plasmid from GW3200	+	+	*leu*$^+$	6.3 (17)	3×10^{-7}
	+	−	*leu*$^+$	0.18 (17)	8×10^{-9}
GC3217 + plasmid from GW3198	+	+	*thi*$^+$	3 (8)	2×10^{-7}

[a] Strains were grown and incubated with or without IPTG, and mutation frequencies were determined as described in Materials and Methods.
[b] Values in parentheses are the numbers of plates analyzed.
[c] Data for IPTG treatment and for no treatment were combined for strains lacking pPS708^{wt}.

DISCUSSION

Some of the strains of *E. coli* containing pPS708^{wt} but not pPS708^{Ala} showed increased mutation after induction with IPTG (Table 5). The levels of both wild-type SspC and SspC^{Ala} increased in response to IPTG (17, 22). However, SspC^{Ala}, unlike the wild-type SspC (12, 17), fails to bind to DNA (22). Since it has been shown that binding of the wild-type SspC to DNA causes a change in DNA configuration (12, 17), we conclude that the mutations we observed were caused by the binding of wild-type protein to the DNA of *E. coli*, thereby altering the DNA configuration. This change in *E. coli* DNA also alters the DNA photochemistry in that the yield of pyrimidine dimers induced by UV radiation is decreased, while the spore photoproduct, a lesion normally not found in *E. coli* cells, is formed (17).

The lack of mutation in Rec$^-$ cells and the filamentation response in some Rec$^+$ cells suggested that the interaction of SspC with DNA induced the SOS system in Rec$^+$ cells. In the case of UV mutagenesis, mutation is believed to take place because of errors in SOS-induced repair mediated by UmuDC proteins (24). Since RecA$^-$ strains do not permit induction of the SOS system (15, 27), the lack of SOS repair renders the Rec$^-$ strains extremely sensitive to UV radiation (8). Upon UV irradiation, these strains do not exhibit UV mutagenesis (24, 28). If the SspC mutations were indeed SOS induced, and thus presumably the SOS system in Rec$^+$ but not Rec$^-$ cells was repairing DNA damage, then we might ask why the Rec$^-$ cells containing pPS708^{wt} were not much more sensitive to killing by IPTG induction (Table 4). One answer to this question might be that SspC does not damage DNA.

The lethal effects of the protein were always evident when there was an increase in mutation (Tables 4, 5, 7, and 9; other data not shown), although we have observed that some pPS708^{wt}-containing cultures of Rec$^+$ strains lose their IPTG-induced mutability while retaining the loss of survival (data not shown). Our hypothesis is that the level of SspC protein in IPTG-induced cells required for mutation may be higher than that required for killing, and we plan to test this idea.

It is unlikely that the lethal effect of the protein resulted from induction of filamentation, since the *sfi* strain was more rather than less sensitive to the protein (Table 4). However, the data obtained with *E. coli umuCD*, *recA*, and *lexA* mutants suggest that the SspC-induced mutations were SOS dependent. Our RecA and UmuDC results together suggest that *umuD* and *umuC* genes are induced by the RecA protein to produce the observed mutations following induction of the SOS response by SspC. Our results with pPS708^{wt} in *umuC36* and *umuD44* mutants (Tables 4 and 9) are similar to those obtained with UV-induced mutation of such mutants (9, 19), namely, a small increase in sensitivity and considerably decreased but still detectable UV-induced mutation. The increased mutation we observed in the strain with the deleted *lexA* gene (Table 10) is in accord with the SOS hypothesis of SspC-induced mutation. However, the data obtained with GC3217 (Tables 5 and 8) do not support the hypothesis, since raising the temperature to 42°C did not produce much increase in pPS708^{wt}-induced mutation (Table 8). We considered that the failure of heat induction of the RecA protein to produce a substantial increase in mutation could have resulted from preferential inactivation of the more heavily mutagenized cells by condensation of nucleoids and compaction of DNA known to be associated with cell killing (17). However, we have not observed any significant difference in the SspC killing of strain GC3217 whether the cells were at 30°C or at 42°C for the first hour of IPTG induction (data not shown). Furthermore, the temperature shift experiment with the parent strain AB1157 (Table 8) showed little or no difference in mutation when the plasmid-containing cells were induced at these two temperatures. The GC3217 data suggest that RecA may play little or no role

TABLE 10. Effect of SspC on mutation in strain JL2491 (*lexA300*)[a]

Presence of pPS708^{wt}	Presence of IPTG	Mutant colonies/ plate[b]	Frequency of mutation
−	+ and −[c]	0.5 (16)	3.5×10^{-8}
+	+	164 (11)	1.2×10^{-5}
+	−	12 (17)	9×10^{-7}

[a] Strains were grown and incubated with or without IPTG, and *leu*$^+$ mutation frequencies were determined as described in Materials and Methods.
[b] Values in parentheses are the numbers of plates analyzed.
[c] Data for IPTG treatment and for no treatment were combined for the strain lacking pPS708^{wt}.

in survival following SspC synthesis, in accord with the small effect of the *recA* mutation on killing (Table 4). However, another possibility is that the extra RecA protein induced in GC3217 at 42°C, in amounts comparable to those of induced SspC (15b, 17a), might have interfered with binding of the SspC protein to DNA, so that even if there was more RecA protein available for mutagenesis, there was a smaller change in DNA conformation. In this case, we would have to assume that the presumed lower binding of SspC to DNA was not sufficient to increase the survival of cells after IPTG induction.

Other data also fail to support the SOS hypothesis of mutation by SspC. The heat-induced SOS system in *recA441* strains stimulated G-C to T-A changes (11). Spontaneous mutation (without pPS708^{wt}) yielded only a minority of such changes in WU3610, with around 3% of the leucine-independent mutations from the amber mutation of WU3610, and a similar minority in pPS708^{wt}-induced mutations (Table 6). The A-T changes, which are apparently much more frequent when the SspC protein has been induced, may be predominantly transitions rather than transversions, since if transitions were not more probable we would expect a higher proportion of transversions causing the Su5 suppressor in ochre revertants and causing the Sul suppressor in amber revertants (Table 6).

The data suggesting that the fraction of total mutations which were A-T base changes was considerably higher in cells containing SspC are of particular interest because it has been observed that, in vitro, SspC binds best to the synthetic polymer poly(dG) · poly(dC), less well to poly(dG-dC), even less well to poly(dA-dT), and not at all to poly(dA) · poly (dT) (17a), probably because of the relative readiness with which these polymers adopt the A conformation. Provided that the synthetic polymer results apply directly to DNA, the Table 6 data may imply that the errors caused by SspC are more likely to occur in A-T regions adjacent to runs of G-C pairs with altered configuration.

It might be assumed that the DNA polymerase made mistakes in the presence of the *B. subtilis* protein because of the altered configuration of the template, and/or that the protein interfered with repair of polymerase errors. However, these ideas do not take into account the SOS effects. We conclude that the SspC lethality does not involve the SOS system, because there is no DNA damage, but that the SspC-induced mutation must be mostly the result of SOS induction, in reaction to the altered DNA caused by SspC binding.

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