

# Inhibition of the *recBCD*-Dependent Activation of Chi Recombinational Hot Spots in SOS-Induced Cells of *Escherichia coli*

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**Nucleotide sequences called Chi (5'-GCTGGTGG-3') enhance homologous recombination near their location by the RecBCD enzyme in *Escherichia coli* (Chi activation). A partial inhibition of Chi activation measured in  $\lambda$  *red gam* mutant crosses was observed after treatment of wild-type cells with DNA-damaging agents including UV, mitomycin, and nalidixic acid. Inhibition of Chi activation was not accompanied by an overall decrease of recombination. A *lexA3* mutation which blocks induction of the SOS system prevented the inhibition of Chi activation, indicating that an SOS function could be responsible for the inhibition. Overproduction of the RecD subunit of the RecBCD enzyme from a multicopy plasmid carrying the *recD* gene prevented the induced inhibition of Chi activation, whereas overproduction of RecB or RecC subunits did not. It is proposed that in SOS-induced cells the RecBCD enzyme is modified into a Chi-independent recombination enzyme, with the RecD subunit being the regulatory switch key.**

The major pathway of homologous recombination in *Escherichia coli* relies on the RecA protein and the RecBCD enzyme (for reviews, see references 8 and 24). The RecBCD enzyme, also called exonuclease V, of *E. coli* and other prokaryotes consists of three different protein subunits coded by the genes *recB*, *recC*, and *recD* (2, 10, 34, 55). Null mutations in the *recB* or *recC* genes of *E. coli* lead to a reduction in many types of recombination events, a loss of repair capacity, and decreased cell viability. In contrast, mutants in the *recD* gene are recombination proficient, are resistant to UV irradiation, and are fully viable, but they lack the exonuclease V activity characteristic for wild-type cells (2, 3, 5). In vitro the RecBCD enzyme has multiple activities, including an ATP-dependent exonuclease for duplex and single-stranded DNA, an ATP-stimulated endonuclease for single-stranded DNA, a DNA-dependent ATPase, and an ATP-dependent DNA helicase (for reviews, see references 45 and 47).

An interesting feature of the RecBCD enzyme is its interaction with the octanucleotide sequence 5'-GCTG GTGG-3', called Chi, which stimulates the *recBCD*-dependent homologous recombination in its vicinity (39, 40, 43). Chi was first recognized in bacteriophage  $\lambda$ . The action of Chi was studied in  $\lambda$  mutants lacking the recombination system Red and the Gam protein, an inhibitor of the *E. coli* RecBCD nuclease. The production of packageable dimeric progeny DNA of such  $\lambda$  mutants depends on the RecA-RecBCD-promoted recombination of the host cell (41), and the progeny yield is low if the phage chromosome does not contain a Chi sequence. Chi sites are present in the *E. coli* chromosome at a density of one per about 5 kb (25) and are active in transduction and conjugation (9). If the RecBCD enzyme encounters the Chi sequence in the appropriate orientation during unwinding of the DNA, it nicks the Chi sequence-containing strand of linear duplex DNA four to six nucleotides before the 3' end of Chi (32, 46). This active orientation of Chi relative to the RecBCD entrance site was previously shown in  $\lambda$  crosses (15). The stimulation of recombination by Chi in vivo and the enzymatic cutting of

DNA near Chi sequences in vitro were reduced or abolished by certain *recB* or *recC* mutations (23, 38) or by mutations in the Chi site (6, 7), suggesting that the cutting is necessary to activate Chi recombinational hot spots. *RecD* mutants are recombination proficient but lack the ability to respond to Chi sequences (2, 5). No enzymatic activity characteristic of the wild-type RecBCD enzyme is detectable in cell extracts (45). The reason for the recombination proficiency of *recD* mutants is not yet understood; it was proposed that in *recD* mutants a recombination pathway, called RecY pathway, different from that in wild-type cells is activated (24). In this pathway the RecBC complex and the *recJ* gene product are probably involved (20, 22). However, the requirement for *recJ* does not apply to  $\lambda$  recombination in *recD* strains (49).

DNA damage or the inhibition of DNA replication in *E. coli* induces the SOS response, which results in the expression of a set of genes involved in diverse functions to improve cellular survival (54). The SOS response is regulated by the *recA* and *lexA* gene products. LexA protein represses the genes of the SOS regulon. After DNA damage, RecA is activated and effects the cleavage of the LexA repressor, which leads to the derepression of the SOS regulon.

Here we report the observation that in cells treated with SOS-inducing agents, including UV irradiation, mitomycin, and nalidixic acid, the *recBCD*-dependent activation of Chi hot spots of recombination is inhibited.

## MATERIALS AND METHODS

**Bacterial strains, phages, and plasmids.** Bacterial and phage strains used are listed in Table 1. AB1157 was transformed with plasmids as previously described (12, 26). The plasmid pDW1 is pBR322 containing a 17-kb *Bam*HI fragment expressing the *thyA*, *recB*, *recC*, *recD*, and *argA* genes of *E. coli* (37). Plasmids pDW11 and pDW12 consist of pBR322 and an 8-kb *Bam*HI-*Cla*I fragment with genes *thyA* and *recC* or a 9-kb *Cla*I-*Bam*HI fragment with genes *recB*, *recD*, and *argA* (55). Plasmid pRR4 is pBR328 containing a 4.1-kb *Cla*I-*Sal*I fragment plus a 2.5-kb *Sal*I fragment expressing the *recB* gene of *E. coli*. The plasmid pPB120 consists of the isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG)-

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TABLE 1. *Escherichia coli* and phage lambda strains

Strain	Genotype (alternate designation)	Source or reference
<i>E. coli</i> strains		
AB1157	<i>thr-1 argE3 proA2 hisG4 thi-1 leuB6 rpsL31 galK2 lacY1 ara-14 xyl-5 mtl-1 tsx-33 supE44</i>	13
WA426	Same as AB1157, but <i>lexA3</i> (DM49)	29
WA429	<i>thr-1 proA2 hisG4 leuB6 galK2 lacY1 rpsL31 recA441 sulA11 lexA51</i> (DM2500)	28
WA470	Same as AB1157, but <i>lexA3</i> <i>recAo98</i> (DM2210)	11
WA576	Same as AB1157, but <i>recF400::Tn5</i>	B. Thoms (52)
WA632	Same as AB1157, but <i>argA+</i> <i>recB21</i>	W. Wackernagel
WA645	Same as AB1157, but $\Delta$ ( <i>pro- lac</i> )XIII <i>recN259::Mu d</i> (Ap <i>lac</i> )	52
WA707	Same as AB1157, but <i>lexA51</i> <i>sfiA11 recA730 srl::Tn10</i> (PC1427)	4
WA721	Same as AB1157, but <i>kdgK51</i> <i>recO1504::Tn5</i> (RDK1541)	22
BT122	Same as AB1157, but <i>recJ284::Tn10</i>	B. Thoms (21)
Phage lambda strains		
$\lambda$ 1081	<i>susJ6 b1453 c1857 <math>\chi^+</math> D123</i>	15, 38
$\lambda$ 1082	<i>susR5 b1453 <math>\chi^+</math> D123</i>	15, 38
$\lambda$ 1083	<i>susJ6 b1453 c1857 <math>\chi^+</math> 76</i>	15, 38
$\lambda$ 1084	<i>susR5 b1453 <math>\chi^+</math> 76</i>	15, 38

inducible expression vector pUC19 (57) and a 3.8-kb *Pst*I fragment with the *recD* gene of *E. coli*. It was obtained from P. Emmerson.

**Media.** LB medium was prepared as previously described (26). If required, media contained ampicillin (40  $\mu$ g/ml), chloramphenicol (20  $\mu$ g/ml), or tetracycline (15  $\mu$ g/ml). Overnight cultures were grown in media supplemented with antibiotics. Log-phase cultures were grown without antibiotics. For lambda crosses cells were grown in media supplemented with 0.2% maltose. The incubation temperature was generally 30°C.

**UV irradiation.** Log-phase cells grown in LB medium were irradiated at room temperature as previously described (51) in phosphate buffer consisting of 40 mM  $\text{Na}_2\text{HPO}_4$ , 22 mM  $\text{KH}_2\text{PO}_4$ , 68 mM NaCl, and 2 mM  $\text{MgSO}_4$ . After irradiation, cells were aerated at 30°C in LB medium.

**Treatment of cells with nalidixic acid and mitomycin.** Cells were treated with nalidixic acid or mitomycin (Sigma Chemical Co.) by adding samples from concentrated stock solutions to log-phase cultures in LB medium and then by aeration at 30°C. The final concentrations depended on the sensitivity of the strain and are stated for each experiment.

**Determination of Chi activation and burst sizes.** Crosses between lambda strains 1081 and 1082 and strains 1083 and 1084 for measurement of Chi activation were performed and evaluated as described previously (38, 42). The multiplicity of infection for each phage was 5 to 7 in all crosses. Values of 1 for Chi activation indicate the absence of recombination

stimulation at Chi sequences (no Chi activation); values greater than 1 indicate recombination stimulation. For measuring  $\text{J}^+\text{R}^+$  recombination frequencies in part of the crosses for Chi activation measurements, unadsorbed phages ( $\leq 25\%$ ) were removed by centrifugation after 20 min of incubation at 30°C for phage adsorption. The recombination frequencies and burst sizes were determined in both Chi crosses; they were roughly equal. The burst sizes in these crosses were generally between 10 and 50. Lower burst sizes (0.4 to 1) were observed in WA632 without and with UV irradiation, in WA426 with 54-J/m<sup>2</sup> UV (size, 1) or 1  $\mu$ g of mitomycin per ml (size, 1 to 2), in WA721 with 27-J/m<sup>2</sup> UV (size, 2), in WA576 with 20-J/m<sup>2</sup> UV (size, 6), in BT122 with 27-J/m<sup>2</sup> UV (size, 4 to 9), and in WA645 with 54-J/m<sup>2</sup> UV (size, 3 to 7).

## RESULTS

**Effects of UV, mitomycin, and nalidixic acid on Chi activation.** The RecBCD enzyme-dependent activation of Chi recombination hot spots was measured by using crosses of  $\lambda$  *red gam* mutant phages (38, 42). In UV-irradiated log-phase cells of AB1157, Chi activation was inhibited. Figure 1A shows the time course of inhibition after different UV doses. At a fluence of 54 J/m<sup>2</sup> (about 10% survival of the cells), the inhibition occurred readily and reached a maximum at 40 to 60 min after irradiation, after which Chi activation recovered during further incubation. Even at a dose of only 18 J/m<sup>2</sup> (survival about 80%) an inhibitory effect was obtained. No inhibition was seen in cells treated identically but not irradiated (Fig. 1A). At a fluence of 108 J/m<sup>2</sup> (survival about 0.1%) recuperation of Chi activation after inhibition was not observed within 2 h. Treatment of cells with nalidixic acid or mitomycin also inhibited Chi activation (Fig. 1B). With these agents the inhibition was maintained after induction, probably because of the permanent presence of the agents in the medium. The substantial reduction of Chi-dependent recombination after treatment with UV, mitomycin, or nalidixic acid was not accompanied by reduction of the total  $\text{J}^+\text{R}^+$  recombination frequency in AB1157 (Table 2). In other *E. coli* K-12 strains, such as K-12s and C600, similar inhibition of Chi activation was observed after UV irradiation as shown for AB1157 (data not shown). *recB*, *recC*, or *recD* null mutants lack the ability for Chi-dependent stimulation of recombination (5, 42). This is manifest in Chi activation values of about 1 as shown for a *recB* mutant (Table 2). In accord with published data (1, 38, 42) for a *recB* mutant (and similarly for a *recC* mutant [data not shown]) the frequency of  $\text{J}^+\text{R}^+$  recombinants was low, as was the burst size (Table 2). UV irradiation of the *recB* mutant did not increase the recombination frequency and the burst size (Table 2), indicating that a pathway of recombination operating independently of *recB* was not induced. It is known that SOS genes are efficiently induced by UV in *recB* mutants (52). The data in Fig. 1 and Table 2 show that in the treated cell populations a complete inhibition of Chi activation was not achieved.

**Inhibition of Chi activation in *lexA* mutants.** Certain *lexA* mutants (e.g., *lexA3*) produce an uncleavable LexA repressor, and therefore the SOS genes are not inducible (18). After treatment with mitomycin or irradiation with a low-UV dose (10% survival) or a high-UV dose (less than 0.01% survival) inhibition of Chi activation was not observed in a *lexA3* mutant (Table 2). This was confirmed in time course experiments (Fig. 1C and D). In strain WA470 (*lexA3 recA o98*), with permanent repression of all SOS genes but with constitutive overproduction of RecA protein because of an

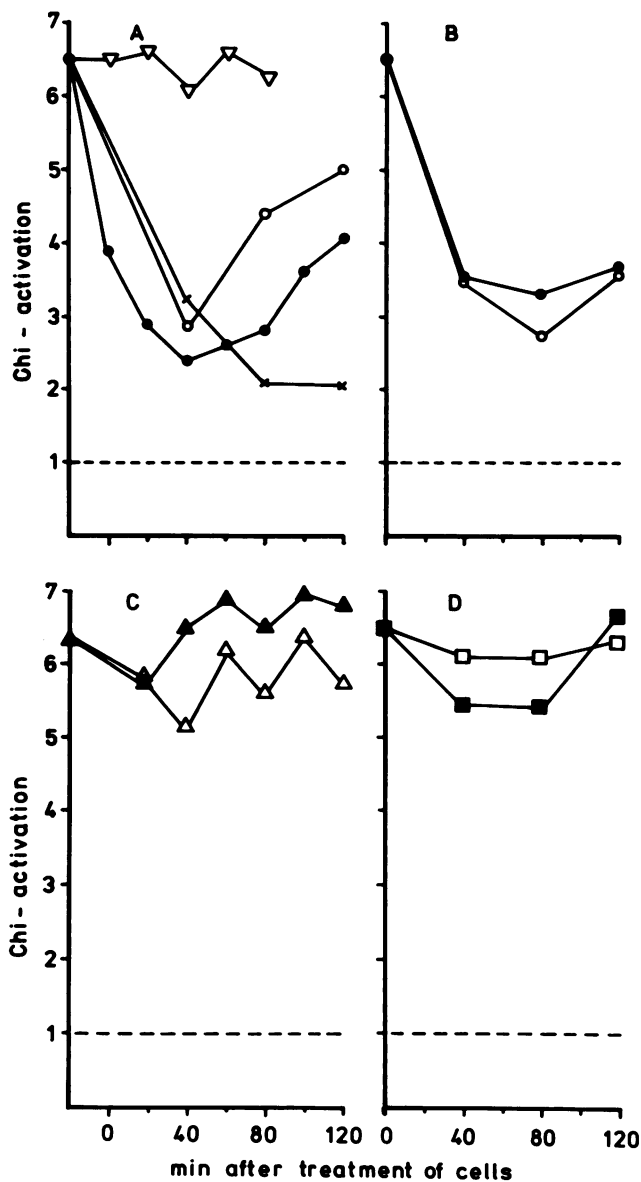


FIG. 1. Chi activation in AB1157 and a *lexA3* mutant after UV irradiation or treatment with mitomycin or with nalidixic acid. Log-phase cells were irradiated with UV or treated with nalidixic acid or mitomycin. They were further aerated at 30°C in LB medium (with 0.2% maltose). After the times indicated, samples were withdrawn and infected with  $\lambda$  phages for the determination of Chi activation as described in Materials and Methods. (A and C) Values to the left of  $t = 0$  show the Chi activation of untreated cells; (B and D) values at  $t = 0$  were obtained with cells removed immediately before application of nalidixic acid or mitomycin. The dashed line indicates absence of Chi activation. (A) AB1157 irradiated with a UV dose of 18 J/m<sup>2</sup> (○), 54 J/m<sup>2</sup> (●), or 108 J/m<sup>2</sup> (×) or unirradiated but otherwise treated identically to the irradiated cells (▽); (B) AB1157 treated with nalidixic acid (10  $\mu$ g/ml; ○) or mitomycin (1  $\mu$ g/ml; ●); (C) WA426 *lexA3* irradiated with a UV dose of 2 J/m<sup>2</sup> (Δ) or 54 J/m<sup>2</sup> (▲); (D) WA426 *lexA3* treated with mitomycin at a concentration of 0.1  $\mu$ g/ml (□) or 1  $\mu$ g/ml (■).

operator mutation, an inhibition of Chi activation without application of SOS-inducing agents was again not observed. Apparently, the limited amount of RecA protein in the *lexA3* mutant was not the reason for the uninducibility of inhibition of Chi activation, but rather the uninducibility was due to the repression of another gene(s). In a *lexA51* mutant the LexA repressor protein is not able to fully repress the SOS genes (28). The Chi activation in uninduced cells of strain WA429 (*lexA51*) was somewhat lower compared with that of AB1157. UV irradiation decreased the level to a value similar to that obtained in UV-irradiated AB1157 cells (Table 2). A similar result (data not shown) was also obtained with a *lexA71::Tn5* mutant (17). The permanent derepression of the SOS regulon was not sufficient to cause inhibition of Chi activation. In strain WA707 (*lexA51 recA730*), with additional constitutive activation of RecA protein the treatment with UV or mitomycin was still required for inhibition of Chi activation (Table 2).

UV-induced inhibition of Chi activation was seen in *recF*, *recJ*, *recN*, and *recO* mutant cells (Table 2), in which the *recF* pathway and other routes of recombination are blocked (19, 24). This suggests that the apparent inhibition of Chi activation is not the result of an induced powerful Chi-independent recombination pathway other than the *recB*- and *recC*-dependent recombination. Inhibition of Chi activation as in strain AB1157 was also observed in UV-irradiated cells of mutants of various SOS genes, including *dinA*, *dinB*, *dinD*, *dinF*, *sulA*, and *umuC* (data not shown). Thus, the gene responsible for damage-induced inhibition of Chi activation remains to be identified.

Chi activation in *E. coli recBCD* deletion strain WA675 with the monocopy plasmid pNE1 carrying the *recBCD* genes of *Serratia marcescens* (34) is almost as high ( $5.9 \pm 0.3$ ) as in the *E. coli* wild type (Table 2). UV irradiation of this strain (54 J/m<sup>2</sup>; about 10% survival) decreased the Chi activation to  $4.5 \pm 0.5$ . In strain WA675, with the *recBCD* genes of *Proteus mirabilis* on pPG35 (55), UV irradiation (54 J/m<sup>2</sup>; about 10% survival) lowered the Chi activation from  $2.9 \pm 0.3$  to  $2.4 \pm 0.2$ . The weaker UV-induced reduction of Chi activation by the RecBCD enzymes of *S. marcescens* and *P. mirabilis* may result from a less efficient interaction of the putative SOS protein of *E. coli* with the RecBCD enzymes of these species. *S. marcescens* is somewhat more closely related to *E. coli* than *P. mirabilis* is to *E. coli* (30).

**Effects of overproduction of the RecBCD enzyme or of its subunits on inhibition of Chi activation.** The UV-induced inhibition of Chi activation was determined in AB1157 derivatives with multicopy plasmids on which all three subunit genes of exonuclease V of *E. coli* were cloned or when only one or two were cloned. Strain AB1157, with the multicopy plasmid pDW1 carrying the *recB*, *recC*, and *recD* genes, has a 20-fold-higher exonuclease activity for double-stranded DNA in crude cell extracts than does AB1157 with pBR322 (37). In this strain, UV irradiation (45 J/m<sup>2</sup>) caused a much weaker inhibition of Chi activation (Table 3) similar to that in AB1157 (Table 2). A high dose of the *recB* plus *recD* genes (plasmid pDW12) or the overproduction of only the *recD* subunit from the pUC19-derived plasmid pPB120 (with or without induction by IPTG) blocked the inhibition of Chi activation after UV irradiation (Table 3). In contrast, multicopy plasmids with only the *recB* (pRR4) or the *recC* (pDW11) gene did not prevent UV-induced inhibition of Chi activation (Table 3). Time course experiments with strains having multiple copies of the *recD*, *recB*, or *recC* gene (Fig. 2A) extend the data of Table 3 and show that Chi activation does not recover in the strain with a high dose of the *recC*

TABLE 2. Effects of various treatments on Chi activation and  $\lambda$  recombination in various *E. coli* strains

Strain (relevant genotype)	Treatment <sup>a</sup>	Chi activation (no. of expts) <sup>b</sup>	J <sup>+</sup> R <sup>+</sup> recombination (%) <sup>c</sup>
AB1157 (wild type)	None	6.7 ± 0.5 (5)	9.3
	UV (54 J/m <sup>2</sup> )	2.3 ± 0.2 (4)	8.1
	MMC (1 µg/ml)	3.3 ± 0.5 (3)	11.5 ± 2.7*
	Nal (10 µg/ml)	2.6 ± 0.2 (3)	4.8 ± 1.2*
WA426 ( <i>lexA3</i> )	None	6.9 ± 0.6 (4)	4.0 ± 0.8
	UV (2 J/m <sup>2</sup> )	6.3 ± 0.9 (3)	3.5
	UV (54 J/m <sup>2</sup> )	6.8 ± 0.2 (3)	2.5
	MMC (0.1 µg/ml)	6.2 ± 0.2 (3)	4.3
	MMC (1 µg/ml)	5.4 (1)	4.5
WA470 ( <i>lexA3 recA98</i> )	None	5.2 ± 0.1 (3)	5.9
	UV (8 J/m <sup>2</sup> )	5.1 ± 0.2 (3)	6.5
WA429 ( <i>lexA51</i> )	None	4.6 ± 0.6 (4)	7.0
	UV (45 J/m <sup>2</sup> )	3.1 ± 0.4 (4)	8.8
WA707 ( <i>lexA51 recA730</i> )	None	5.8 ± 0.1 (3)	6.8
	UV (54 J/m <sup>2</sup> )	2.9 ± 0.2 (3)	7.9
WA576 ( <i>recF400::Tn5</i> )	None	5.4 ± 0.2 (3)	9.8
	UV (20 J/m <sup>2</sup> )	2.8 ± 0.3 (3)	9.5
BT122 ( <i>recJ284::Tn10</i> )	None	5.4 ± 0.1 (4)	7.6 ± 0.5
	UV (27 J/m <sup>2</sup> )	2.5 ± 0.3 (4)	8.3 ± 0.4
WA721 ( <i>recO1504::Tn5</i> )	None	5.4 (2)	9.1 ± 0.6
	UV (27 J/m <sup>2</sup> )	3.0 (2)	8.5 ± 1.0
WA645 ( <i>recN259</i> )	None	5.3 (2)	9.2 ± 2.0
	UV (54 J/m <sup>2</sup> )	2.7 (2)	7.6 ± 1.5
WA632 ( <i>recB21</i> )	None	1.1 ± 0.2 (4)	0.5 ± 0.1
	UV (5 J/m <sup>2</sup> )	1.0 ± 0.1 (4)	0.4 ± 0.1

<sup>a</sup> The UV doses were chosen to give about 10% survival; an exception is the UV dose of 54 J/m<sup>2</sup> for strain WA426. The period of postirradiation incubation was 50 to 60 min. Mitomycin (MMC) and nalidixic acid (Nal) were used in the concentrations given. The period of incubation with MMC and Nal was 60 to 80 min.

<sup>b</sup> Experiments done with centrifugation and without were combined, because their results were similar.

<sup>c</sup> The recombination frequencies were determined only in experiments in which unadsorbed phages were removed by centrifugation, except for the experiments marked with asterisks, in which centrifugation was omitted. Values are means of two crosses; values with standard deviation are means of at least four crosses. The burst sizes of the crosses in WA632 were only between 0.4 and 1 and were not affected by the UV irradiation.

gene within 2 h after irradiation. The J<sup>+</sup>R<sup>+</sup> recombination frequencies were not affected by the UV irradiation of the three strains whether or not Chi activation was inhibited (Fig. 2B).

## DISCUSSION

The treatment of *E. coli* cells with DNA-damaging agents, including UV light, mitomycin, and nalidixic acid at doses and incubation conditions which induce the SOS regulon resulted in an inhibition of the *recBCD*-dependent stimulation of homologous recombination at Chi sequences (Fig. 1 and Table 2). The inhibition did not occur in a *lexA3* mutant in which the uncleavable Lex protein prevents the damage-induced derepression of the SOS regulon. This suggested that an SOS function is required to cause the inhibition. Also, the time course of appearance of the inhibition after application of the inducing treatments (Fig. 1) is typical for the derepression of SOS genes (27). In cells with a permanently derepressed SOS regulon due to a defective LexA repressor (*lexA51*), a DNA-damaging treatment was still required to obtain inhibition. This points to a specific role of DNA damage. This role cannot be solely the activation of the RecA protein to become a coprotease, because in a

TABLE 3. Effects of overproduction of RecBCD enzyme or subunits of the enzyme on Chi activation

<i>E. coli</i> strain	Relevant genes on plasmids	Treatment (J/m <sup>2</sup> ) <sup>a</sup>	Chi activation (no. of expts)
AB1157 pDW1	<i>recBCD</i>	None	6.7 ± 0.7 (4)
		UV (45)	4.4 ± 0.2 (4)
AB1157 pPB120	<i>recD</i>	None	7.1 ± 0.8 (4) <sup>b</sup>
		UV (45)	6.0 (2)
		UV (45) + IPTG <sup>c</sup>	6.4 ± 0.8 (4)
AB1157 pDW12	<i>recBD</i>	None	5.7 (2)
		UV (54)	5.9 (2)
AB1157 pRR4	<i>recB</i>	None	5.4 ± 0.1 (3)
		UV (54)	2.6 ± 0.1 (3)
AB1157 pDW11	<i>recC</i>	None	3.7 ± 0.2 (3)
		UV (54)	2.6 ± 0.3 (3)

<sup>a</sup> The UV dose was chosen to give about 10% survival. The period of postirradiation incubation was 50 to 60 min.

<sup>b</sup> In control experiments AB1157 pUC19 gave a Chi activation value of 5.3 without UV and of 1.9 with UV (54 J/m<sup>2</sup>) or 2.6 with UV and 1 mM IPTG.

<sup>c</sup> IPTG (1 mM) was applied to induce the overexpression of *recD* on the pUC19-derived plasmid pPB120.

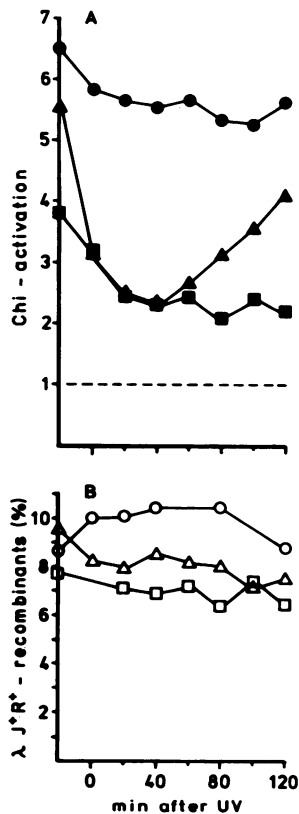


FIG. 2. Chi activation (A) and  $\lambda$  recombination (B) after UV irradiation of AB1157 strains with multicopy plasmids carrying the *recB*, the *recC*, or the *recD* gene. Log-phase cells were irradiated with UV, and after the times indicated, the Chi activation (A) and the  $J^+R^+$  recombinant frequency were determined. For details, see the legend to Fig. 1. The strains were AB1157 pPB120 (*recD*) with 45  $J/m^2$  and 1 mM IPTG (●, ○), AB1157 pRR4 (*recB*) with 54  $J/m^2$  (▲, △), and AB1157 pDW11 (*recC*) with 54  $J/m^2$ .

mutant with permanently activated RecA protein (*recA730*), DNA damage also increased the inhibition of Chi activation. The requirement of SOS derepression plus DNA damage is probably why in earlier experiments normal Chi-stimulated recombination was observed, although the use of a *dnaBts* mutant at elevated temperature induced the SOS response (16, 44). It may be noted that the damage-inducible *dnaA* gene requires wild-type alleles of *recA* and *lexA* for induction but is not constitutively expressed in a *lexA::Tn5* (Def) *recA730* background (33).

Overproduction of RecBCD enzyme counteracted the induced inhibition and indicated that the inhibition of Chi activation acts on a *recBCD*-dependent step. Specifically, from the observation that the presence of extra *recD* gene copies in the cell blocked the inhibition of *recBCD*-dependent Chi activation (Table 3; Fig. 2), it is concluded that the inhibition is caused by the interaction of a presumptive SOS protein with the RecD protein either free or within the RecBCD enzyme. (Because large amounts of RecD protein overcome the inhibitory effect, we assume that the interaction is noncatalytic.) Thus, SOS-induced cells may harbor RecBCD enzyme molecules having an inactive RecD subunit or lacking the RecD subunit. If such modified RecBCD enzyme molecules (RecBCD\*) contribute no longer to Chi activation but still can efficiently promote recombination,

then this would explain the inhibition of Chi activation without reduction of gross recombination. It is known that the RecBC enzyme in *recD* mutants performs recombination efficiently but independently of Chi sequences (2, 5). On the other hand, lack of all activities of the RecBCD enzyme, e.g., as in *recB* or *recC* null mutants, eliminates Chi activation and drastically reduces gross recombination in  $\lambda$  *red gam* mutant crosses (1, 38, 42) (Table 2). This was not observed in the SOS-induced cells. It has recently been shown that recombination proficiency and Chi activation are also not correlated in many *recBCD* mutants (1). Explanations in which the unknown SOS protein would indirectly affect the RecD protein or its function are also possible. Extra *recD* gene copies in wild-type cells with single *recB* and *recC* genes do not increase the level of exonuclease V activity for double-stranded DNA (36) or recombination proficiency (Fig. 2).

In our experiments Chi activation was never completely absent upon SOS induction (Fig. 1 and 2; Tables 2 and 3). Perhaps not all cells were induced or the induction occurred nonsynchronously in the cell populations employed in the experiments. Alternatively, incompletely inhibited Chi activation may indicate that within the individual SOS-induced cells RecBCD\* enzyme molecules together with normal RecBCD enzymes are present. We think that the second explanation is more likely. Perhaps the induced amount of the presumptive SOS protein does not fully titrate the RecD or RecBCD proteins. Thus, in an induced cell recombination events may be performed by RecBCD and by RecBCD\* enzymes side by side. The level of residual Chi activation would then result from the balance between RecBCD and RecBCD\* enzymes. Extra RecD subunits provided by a high *recD* gene dose appear to shift the balance towards normal RecBCD enzyme molecules.

Interestingly, in uninduced cells with an overproduction of the RecC subunit (AB1157 pDW11), Chi activation was lower than in AB1157 (Table 3). Probably this is because of an interaction between the RecC and the RecD protein subunits. Since certain *recC* mutants (*recC‡*) were phenotypically indistinguishable from *recD* mutants it was proposed that in *recC‡* mutants the RecD protein does not bind to the altered RecC protein as it does in wild-type cells (2, 5). In the RecC-overproducing strain AB1157 pDW11 part of the RecD protein molecules may complex to free RecC subunits present in excess over RecB and RecD proteins. For each RecC-RecD complex formed (inactive in recombination), one RecB-RecC can form which is Rec<sup>+</sup> but does not activate Chi. Thus, overexpression of RecC would decrease the number of RecBCD enzyme molecules and increase that of RecBC molecules. This would lead to a lower Chi activation in the cells. This was observed (Table 3) and lends support to the proposal of the RecC-RecD interaction. Further, the observation that in untreated cells with RecB overproduction (AB1157 pRR4) Chi activation was not reduced is consistent with the assumption that RecB has no site for interaction with RecD.

Our experiments do not provide an answer to the question of whether the RecBCD\* enzyme has lost the double-strand exonuclease activity of the RecBCD enzyme, although this is an intriguing possibility. It has previously been postulated that an inhibitor of the exonuclease V activity is induced in cells following DNA damage as part of the *recA-lexA*-controlled SOS response (for reviews, see references 53 and 56) or independently of *lexA* (14). The latter possibility is excluded as an explanation of our observations because the inhibition of Chi activation did not occur in *lexA3* cells. It is

conceivable that an SOS protein uses the RecD protein as a switch key to change the RecBCD enzyme having strong duplex DNA exonuclease activity to a moderate enzyme with low or no degradative activity towards duplex DNA ends but with high recombinogenic potential. Further work is necessary to prove or disprove such a concept. Recently, evidence has been provided that the RecBC enzyme devoid of RecD subunit has in vitro no duplex DNA exonuclease activity (31, 45) but is involved in vivo in DNA unwinding which probably makes the enzyme a potent recombinase (35).

We do not yet understand details of the mechanism of inhibition of the Chi activation. The RecD subunit, which is essential for the duplex DNA exonuclease activity and for the Chi activation function of the RecBCD enzyme, apparently plays a central role as the target of the inhibitory action. In this context it is interesting to note that in a recent hypothesis (48, 50) and variant hypotheses on the interaction of the RecBCD enzyme with Chi sequences it was proposed that during DNA unwinding by the RecBCD enzyme the RecD subunit is removed from the enzyme complex at Chi, thereby switching on the recombination-promoting activity of the enzyme. Observations supporting this hypothesis have been provided (44, 49).

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