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′-GCTG GTGG-3′) enhance homologous recombination near their location by the RecBCD enzyme in Escherichia coli (Chi activation). A partial inhibition of Chi activation measured in λ 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 lexA 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 inhibited activation of Chi, 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 procaryotes 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 λ. The action of Chi was studied in λ 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 λ 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 λ 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 λ 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 affects 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 BamHI fragment expressing the thyA, recB, recC, recD, and arg genes of E. coli (37). Plasmids pDW11 and pDW12 consist of pBR322 and an 8-kb BamHI-ClaI fragment with genes thyA and recC or a 9-kb ClaI-BamHI fragment with genes recB, recD, and argA (55). Plasmid pR4 is pBR328 containing a 4.1-kb ClaI-SalI fragment plus a 2.5-kb SalI fragment expressing the recB gene of E. coli. The plasmid pB120 consists of the isopropyl-β-D-thiogalactopyranoside (IPTG)-
stimulation at Chi sequences (no Chi activation); values greater than 1 indicate recombination stimulation. For measuring J+R+ recombination frequencies in part of the crosses for Chi activation measurements, unasorbed phages (±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/m² UV (size, 1) or 1 ìg of mitomycin per ml (size, 1 to 2), in WA721 with 27-J/m² UV (size, 2), in WA576 with 20-J/m² UV (size, 6), in BT122 with 27-J/m² UV (size, 4 to 9), and in WA645 with 54-J/m² 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 λ 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/m² (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/m² (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/m² (survival about 0.1%) recovery 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 J+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 J+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 unseparable 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 recA98), with permanent repression of all SOS genes but with constitutive overproduction of RecA protein because of an

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**TABLE 1. Escherichia coli and phage lambda strains**

| Strain     | Genotype (alternate designation) | Source or reference |
|------------|----------------------------------|---------------------|
| **E. coli strains** |                                 |                     |
| AB1157     | thr-1 argE3 proA2 hisG4 thi-l   | 13                  |
|            | leuB6 rpsL31 galK2 lacY1         |                     |
|            | ara-14 xyl-5 mtl-1 tss-33        |                     |
|            | supE4                            |                     |
| WA426      | Same as AB1157, but lecA3        | 29                  |
|            | (DM49)                           |                     |
| WA429      | thr-1 proA2 hisG4 leuB6          | 28                  |
|            | galK2 lacY1 rpsL31               |                     |
|            | recA441 suA11 lexA51            | (DM2500)            |
| WA470      | Same as AB1157, but lecA3        | 11                  |
|            | recAo98 (DM2210)                 |                     |
| WA576      | Same as AB1157, but recF400::Tn5 | B. Thoms (52)       |
| WA632      | Same as AB1157, but arg4*        | W. Wackernagel      |
| WA645      | Same as AB1157, but Δ(pro-lac)   | 52                  |
|            | lacXII recN59::Mu d(Ap lac)      |                     |
| WA707      | Same as AB1157, but lexA51       | 4                   |
|            | sflA11 recA730 srl::Tn10         | (PC1427)            |
| WA721      | Same as AB1157, but kdgK51       | 22                  |
|            | recO1504::TnlO                   |                     |
| BT122      | Same as AB1157, but recJ284::Tn10| B. Thoms (21)       |
| **Phage lambda strains** |                                 |                     |
| λ 1081     | susIo b1453 c1857 x+ D123        | 15, 38              |
| λ 1082     | susIo b1453 x+ D123              | 15, 38              |
| λ 1083     | susIo b1453 c1857 x+ 76          | 15, 38              |
| λ 1084     | susIo b1453 x+ 76                | 15, 38              |
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 lexA31 mutant the LexA repressor protein is not able to fully repress the SOS genes (28). The Chi activation in uninduced cells of strain WA429 (lexA31) 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 (lexA31 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 mutants (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 ± 0.3) as in the E. coli wild type (Table 2). UV irradiation of this strain (54 J/m²; about 10% survival) decreased the Chi activation to 4.5 ± 0.5. In strain WA675, with the recBCD genes of Proteus mirabilis on pPG35 (55), UV irradiation (54 J/m²; about 10% survival) lowered the Chi activation from 2.9 ± 0.3 to 2.4 ± 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 with 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/m²) 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.
The burst marked with mm. gene three strains (Fig. 2B). Also, the in that induced and Table DNA application the RecA a

| Strain (relevant genotype) | Treatment* | Chi activation (no. of expts)* | J⁺R⁺ recombination (%)² |
|---------------------------|------------|-------------------------------|-------------------------|
| AB1157 (wild type)        | None       | 6.7 ± 0.5 (5)                 | 9.3                     |
|                           | UV (54 J/m²) | 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 (lexA3)             | None       | 6.9 ± 0.6 (4)                 | 4.0 ± 0.8               |
|                           | UV (2 J/m²)  | 6.3 ± 0.9 (3)               | 3.5                     |
|                           | UV (54 J/m²) | 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 (lexA3 recAo98)     | None       | 5.2 ± 0.1 (3)                | 5.9                     |
|                           | UV (8 J/m²)  | 5.1 ± 0.2 (3)               | 6.5                     |
| WA429 (lexA51)            | None       | 4.6 ± 0.6 (4)                | 7.0                     |
|                           | UV (45 J/m²) | 3.1 ± 0.4 (4)            | 8.8                     |
| WA707 (lexA51 recA730)    | None       | 5.8 ± 0.1 (3)                | 6.8                     |
|                           | UV (54 J/m²) | 2.9 ± 0.2 (3)            | 7.9                     |
| WA576 (recF400::Tn5)      | None       | 5.4 ± 0.2 (3)                | 9.8                     |
|                           | UV (20 J/m²) | 2.8 ± 0.3 (3)            | 9.5                     |
| BT122 (recJ284::Tn10)     | None       | 5.4 ± 0.1 (4)                | 7.6 ± 0.5               |
|                           | UV (27 J/m²) | 2.5 ± 0.3 (4)            | 8.3 ± 0.4               |
| WA721 (recO1504::Tn5)     | None       | 5.4 (2)                      | 9.1 ± 0.6               |
|                           | UV (27 J/m²) | 3.0 (2)                   | 8.5 ± 1.0               |
| WA645 (recN259)           | None       | 5.3 (2)                      | 9.2 ± 2.0               |
|                           | UV (54 J/m²) | 2.7 (2)                   | 7.6 ± 1.5               |
| WA632 (recB21)            | None       | 1.1 ± 0.2 (4)                | 0.5 ± 0.1               |
|                           | UV (5 J/m²)  | 1.0 ± 0.1 (4)              | 0.4 ± 0.1               |

* The UV doses were chosen to give about 10% survival; an exception is the UV dose of 54 J/m² 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.

* Experiments done with centrifugation and without were combined, because their results were similar.

* 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⁺R⁺ 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

| E. coli strain | Relevant genes on plasmids | Treatment (J/m²)⁴ | Chi activation (no. of expts) |
|---------------|--------------------------|-------------------|-------------------------------|
| AB1157 pDW1   | *recBCD*                 | None              | 6.7 ± 0.7 (4)                |
|               |                          | UV (45)           | 4.4 ± 0.2 (4)                |
| AB1157 pPB120 | *recD*                   | None              | 7.1 ± 0.8 (4)⁵              |
|               |                          | UV (45)           | 6.0 (2)                      |
|               |                          | UV (45) + IPTG⁵   | 6.4 ± 0.8 (4)                |
| AB1157 pDW12  | *recBD*                  | None              | 5.7 (2)                      |
|               |                          | UV (54)           | 5.9 (2)                      |
| AB1157 pRR4   | *recB*                   | None              | 5.4 ± 0.1 (3)                |
|               |                          | UV (54)           | 2.6 ± 0.1 (3)                |
| AB1157 pDW11  | *recC*                   | None              | 3.7 ± 0.2 (3)                |
|               |                          | UV (54)           | 2.6 ± 0.3 (3)                |

* The UV dose was chosen to give about 10% survival. The period of postirradiation incubation was 50 to 60 min.

* In control experiments, AB1157 pUC19 gave a Chi activation value of 5.3 without UV and of 1.9 with UV (54 J/m²) or 2.6 with UV and 1 mM IPTG.

* IPTG (1 mM) was applied to induce the overexpression of *recD* on the pUC19-derived plasmid pPB120.
then this would explain the inhibition of Chi activation without reduction of gross recombination. It is known that the RecBCD 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 λ 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 (recC4) were phenotypically indistinguishable from recD mutants it was proposed that in recC4 mutants the RecD protein does not bind to the altered RecC protein as it does in wild-type cells (2, 55). 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* 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
clones. which probably makes that of VOL. duplex switchkey conceivable (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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