

Genetic Evidence for the Requirement of RecA Loading Activity in SOS Induction after UV Irradiation in *Escherichia coli*

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The SOS response in *Escherichia coli* results in the coordinately induced expression of more than 40 genes which occurs when cells are treated with DNA-damaging agents. This response is dependent on RecA (coprotease), LexA (repressor), and the presence of single-stranded DNA (ssDNA). A prerequisite for SOS induction is the formation of a RecA-ssDNA filament. Depending on the DNA substrate, the RecA-ssDNA filament is produced by either RecBCD, RecFOR, or a hybrid recombination mechanism with specific enzyme activities, including helicase, exonuclease, and RecA loading. In this study we examined the role of RecA loading activity in SOS induction after UV irradiation. We performed a genetic analysis of SOS induction in strains with a mutation which eliminates RecA loading activity in the RecBCD enzyme (*recB1080* allele). We found that RecA loading activity is essential for SOS induction. In the *recB1080* mutant RecQ helicase is not important, whereas RecJ nuclease slightly decreases SOS induction after UV irradiation. In addition, we found that the *recB1080* mutant exhibited constitutive expression of the SOS regulon. Surprisingly, this constitutive SOS expression was dependent on the RecJ protein but not on RecFOR, implying that there is a different mechanism of RecA loading for constitutive SOS expression.

The SOS response in *Escherichia coli* results in the simultaneously induced expression of more than 40 genes which occurs when cells are treated with DNA-damaging agents (7; for a review see reference 8). The elevated expression of these genes increases the capacity of cells for DNA repair, damage tolerance, DNA replication, and mutagenesis (29, 39). Historically, it is known that the SOS response is dependent on *recA* and *lexA* gene products, as well as on the presence of single-stranded DNA (ssDNA). The LexA protein is the repressor of the SOS regulon which binds to specific sequences, called SOS boxes, that are present in the promoter regions of SOS genes (15, 16, 17). LexA is responsible for the very weak basal expression of the SOS genes under normal physiological conditions. SOS induction occurs when activated RecA protein causes the self-cleavage of the LexA repressor (for reviews see references 23 and 36). The amounts of the RecA, LexA, RecN, DNA Pol II, UvrABC, RuvABC, UmuDC, and SfiA proteins, among other proteins, increase during SOS induction and influence various aspects of DNA metabolism.

A critical step in the SOS signaling mechanism is the production of a RecA-ssDNA filament, which is important for activation of the RecA protein. The RecA-ssDNA filament can be used for homologous recombination and SOS induction (for a review see reference 23). There are three essential enzymatic activities that are required for the formation of a RecA-ssDNA filament from double-stranded DNA (dsDNA) ends: helicase, 5'-3' exonuclease, and RecA loading onto 3'-ssDNA. These activities are linked to the proteins of the two main recombination mechanisms in *E. coli*, RecBCD and RecFOR. The

RecBCD enzyme has all three enzymatic activities, whereas in the RecFOR mechanism there are different proteins for different activities, including RecQ (helicase), RecJ (nuclease), and the RecFOR complex (RecA loading). The RecFOR recombination mechanism can process dsDNA ends only in a *recBC sbcBC(D)* multiple mutant, which contains an inactivated RecBCD enzyme, exonuclease I, and SbcCD nuclease (for a review see reference 14). In wild-type (wt) cells the RecFOR mechanism is involved in processing of single-strand gaps (SSGs) which occur in daughter strands after reinitiation of DNA replication downstream from a noncoding lesion (9, 14). The most important role in this process is that of the RecFOR complex itself, which replaces the single-strand DNA binding (SSB) protein complexed with ssDNA with the RecA protein (25). Additional elements of the RecFOR pathway (RecQ helicase and RecJ nuclease) may help in the production of more ssDNA from the original SSG to produce a longer filament.

Analogous to recombination, there are two pathways for SOS induction, the RecBCD and RecFOR pathways, which exhibit substrate specificity for dsDNA ends (or breaks) and SSGs, respectively (4, 21, 23). In wt cells, SOS induction immediately after UV irradiation is dependent on RecFOR proteins. However, when dsDNA ends appear later, as a result of nucleotide excision repair and replication fork collapse, SOS induction requires the RecBCD enzyme as well. This is reflected in the fact that SOS induction after UV irradiation in *recFOR* mutants is not completely eliminated but is delayed (10, 35, 38) and is dependent on the RecBCD enzyme (30).

The requirement for both RecBCD and RecFOR in SOS induction was revealed using null mutants whose mutations which completely eliminated the functions of the mutated gene products. In this study we focused on the RecA loading activity of the RecBCD enzyme in vivo and its role in the SOS signal-

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ing mechanism. We expected that elimination of RecA loading activity by appropriate mutations would have serious implications for SOS induction. To test the effect of a mutation which specifically eliminated RecA loading ability, we used the *recB1080* allele (13, 40). The point mutation in this allele is located in the nuclease domain of the RecBCD enzyme (RecB^{nuc}), which is also involved in RecA binding (33). Consequently, the RecB1080CD form of the enzyme is nuclease deficient and is unable to load RecA protein onto ssDNA, but it still has functional helicase activity (2, 37, 40).

In this study we compared the levels of SOS induction after UV irradiation in strains in which one or both mechanisms of RecA loading activity were inactivated. We found that RecA loading activity is essential for SOS induction. SOS induction in the *recB1080* mutant is independent of the RecQ helicase and is partially dependent on the RecJ nuclease. We also found that the *recB1080* mutant exhibits constitutive expression of the SOS regulon, which is eliminated by *recJ* and *recD* mutations. Surprisingly, *recFOR* mutations have no effect on constitutive SOS expression.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. The bacterial strains used in this study are shown in Table 1. The N5071, TRM452, and TRM387 strains were kindly provided by R. G. Lloyd, University of Nottingham, Nottingham, England. Transduction was carried out as described by Miller (24).

Media and growth conditions. Bacteria were grown in high-salt Luria broth at 37°C with aeration to the early log phase (optical density at 600 nm, ~0.2) and then used for UV irradiation and β -galactosidase assays.

UV irradiation. After the cultures reached the early log phase, 10 ml of each culture was centrifuged, and the cells were resuspended in 10 ml of M/15 phosphate buffer. UV irradiation was performed by using a 50-J/m² dose from a Hoefer UVC 500 UV cross-linker (Amersham Biosciences). After irradiation the cells were centrifuged, resuspended in 10 ml of high-salt Luria broth, and then incubated for 180 min at 37°C. The bacteria were irradiated at room temperature.

Measurement of β -galactosidase activity. In the bacterial strains used for the β -galactosidase assay *lacZ* was fused to the *sfiA* regulatory region, and induction of the SOS response was assayed by determining the β -galactosidase activity as described by Miller (24); the results were expressed in Miller units. SOS induction was also described by using the induction factor (IF), which is the ratio of the β -galactosidase activity in UV-irradiated cells to the β -galactosidase activity in the control cells at a given time.

RESULTS

RecA loading activity is essential for SOS induction. To test specifically whether RecA loading activity is required for SOS induction, we used strains with separately inactivated RecFOR-mediated RecA loading and RecBCD-mediated RecA loading, as well as strains in which both mechanisms of RecA loading were inactivated. All strains had a *lacZ* insertion downstream of the regulatory region of *sfiA*, a gene which belongs to the SOS regulon. The levels of *lacZ* expression were assayed by determining the β -galactosidase activity (expressed in Miller units); this activity was proportional to the level of SOS induction. Another measure of SOS induction is the induction factor, which is the ratio of the *lacZ* expression in cells treated with UV irradiation to the *lacZ* expression in cells not treated with UV irradiation.

Figure 1 shows the levels of SOS expression in the wt strain and *recB268*, *recB1080*, and *recO* mutants. The results are expressed in Miller units (β -galactosidase units) for prepara-

tions that received and did not receive UV treatment (Fig. 1A) and as IFs (Fig. 1B). The highest level of SOS expression after UV irradiation was that of the *recB1080* mutant, and the maximum value was more than 1,900 (β -galactosidase units). The level of SOS expression after UV irradiation in wt cells was lower (maximum value, ~1,600 β -galactosidase units), but it was comparable to that of the *recB1080* mutant. On the other hand, the levels of SOS expression after UV irradiation in the *recB268* (maximum value, ~1,400 β -galactosidase units) and *recO* (maximum value, ~1,000 β -galactosidase units) mutants were lower than the levels of SOS expression in the wt strain and the *recB1080* mutant.

An important feature of the *recB1080* mutant was that it had an increased basal level of SOS expression (maximum value, ~1,100 β -galactosidase units) which was considerably higher than the basal levels of SOS expression in the wt strain (maximum value, ~170 β -galactosidase units), the *recO* mutant (maximum value, ~300 β -galactosidase units), and the *recB268* mutant (maximum value, ~150 β -galactosidase units) (Fig. 1A). However, the IFs were greatest for the *recB268* (*recB* null) mutant (IF, ~26) and wt cells (IF, ~18), in which the SOS induction started immediately after UV irradiation (Fig. 1B). The reason for greater IFs for the *recB268* mutant and wt cells was that the *recB1080* single mutant had constitutive subinduction (high level of SOS expression without UV irradiation). In contrast, the *recO* single mutant showed a typical delayed SOS response that resulted in a maximum IF of around 3.5. The *recB1080* single mutant showed levels of SOS induction (IF, around 3.5) similar to those of the *recO* mutant, but induction started immediately after UV irradiation (Fig. 1B).

Figure 2A shows the SOS induction (expressed in β -galactosidase units) in a *recB1080 recO* strain in which both mechanisms of RecA loading were eliminated. The *recO* mutation reduced SOS induction in the *recB1080* genetic background to the basal level of SOS expression in the *recB1080* single mutant. This was consistent with our expectation that RecA loading activity is crucial for SOS induction. This decrease in SOS expression was due to the effect of UV irradiation rather than to the effect on constitutive SOS expression. Similar results were obtained with *recB1080 recF* and *recB1080 recR* double mutants (data not shown). To further test whether RecA loading activity is essential for the SOS signaling mechanism, we monitored SOS induction in a *recB1080 recD* double mutant and in a *recB1080 recD recO* triple mutant. It is known that RecB1080C(D⁻), an enzyme produced by *recB1080 recD* cells, possesses RecA loading activity due to inactivation of the RecD subunit, which is an inhibitor of RecA loading (1). On the basis of this knowledge, we expected that inactivation of *recO* in a *recB1080 recD* genetic background would allow delayed SOS induction to occur. The SOS induction in the *recB1080 recD recO* triple mutant was less than that in the *recB1080 recD* double mutant and was similar to the SOS expression in a *recB1080 recO* double mutant. It should be emphasized that the *recD* mutation reduced the basal level of SOS expression in a *recB1080* background (Fig. 2B). However, the level of β -galactosidase activity after UV irradiation was considerably higher than the basal level, suggesting that the *recB1080 recD recO* strain was able to induce an SOS response. The results shown in Fig. 2A and B are expressed as IFs in Fig. 2C. When both mechanisms of RecA loading were eliminated

TABLE 1. *E. coli* K-12 strains used in this study

Strain	Relevant genotype	Reference or source
Bacterial strains related to AB1157		
AB1157	F ⁻ <i>thr-1 leuB6 Δ(gpt-proA)62 hisG4 thi-1 argE3 lacY1 galK2 ara-14 xyl-5 mtl-1 tsx-33 supE44 rpsL31 kdgK51 rfbD1 mgl-51 λ⁻ rac</i>	3
AM208	AB1157 <i>recR256::Tn5</i>	R. G. Loyd
N5170	AB1157 <i>thr⁺ leu⁺ Δ(pro-lac) sfiA::Mud (Ap lac MuB::Tn9)</i>	R. G. Loyd
N3071	AB1157 <i>recB268::Tn10</i>	R. G. Loyd
N3072	AB1157 <i>recA269::Tn10</i>	R. G. Loyd
RIK174	AB1157 <i>recB1080</i>	13
RIK123	AB1157 <i>recB1067</i>	13
RIK144	AB1157 <i>recD1903::Tn10d(Tet)</i>	13
RIK155	AB1157 <i>recD1903::Tn10d(Tet) recB1067</i>	13
IRB103	AB1157 <i>recO1504::Tn5</i>	27
IIB360	AB1157 (<i>recB1080</i>) <i>argA::Tn10</i>	P1.N5071 × RIK174
IIB361	AB1157 (<i>recB1067</i>) <i>argA::Tn10</i>	P1.N5071 × RIK123
IIB290	AB1157 <i>recB1080 recD1903::Tn10d(Tet)</i>	P1.RIK144 × RIK174
IIB294	AB1157 <i>recF400::Tn5</i>	P1.WA576 × AB1157
LMM1032	AB1157 <i>recJ2052::Tn10kan</i>	D. Zahradka
LMM1215	AB1157 <i>ΔrecQ::kan</i>	D. Zahradka
WA576	AB1157 <i>recF400::Tn5</i>	W. Wackernagel
Bacterial strains related to MG1655		
MG1655	F ⁻ <i>rec⁺</i> (wt)	
N5071	MG1655 <i>argA::Tn10</i>	R. G. Lloyd
TRM452	<i>Δlac ΔattB::P_{BAD}I-SceI</i>	R. G. Lloyd
TRM387	<i>ΔargE::I-SceIcs::cat ΔattB::P_{BAD}I-SceI</i>	22
IIB385	<i>Δlac ΔattB::P_{BAD}I-SceI ΔargE::I-SceIcs::cat</i>	P1.TRM387 × TRM452
IIB386	<i>Δlac ΔattB::P_{BAD}I-SceI ΔargE::I-SceIcs::cat sfiA::Mud (Ap lac MuB::Tn9)</i>	P1.5170 × IIB385
IIB388	As IIB386, <i>recB1080 argA::Tn10</i>	P1.IIB360 × IIB386
IIB389	As IIB386, <i>recB1067 argA::Tn10</i>	P1.IIB361 × IIB386
IIB390	As IIB386, <i>recB1080 recD1903::Tn10d(Tet)</i>	P1.IIB290 × IIB386
IIB391	As IIB386, <i>recB1067 recD1903::Tn10d(Tet)</i>	P1.RIK155 × IIB386
IIB392	As IIB386, <i>recO1504::Tn5</i>	P1.IRB103 × IIB386
IIB393	As IIB386, <i>recB1080 recD1903::Tn10d(Tet) recO1504::Tn5</i>	P1.IRB103 × IIB390
IIB394	As IIB386, <i>recB1067 recD1903::Tn10d(Tet) recO1504::Tn5</i>	P1.IRB103 × IIB391
IIB395	As IIB386, <i>recD1903::Tn10d(Tet)</i>	P1.RIK144 × IIB386
IIB401	As IIB386, <i>recB1080 recO1504::Tn5</i>	P1.IRB103 × IIB388
IIB402	As IIB386, <i>recB1067 recO1504::Tn5</i>	P1.IRB103 × IIB389
IIB407	As IIB386, <i>ΔrecQ::kan</i>	P1.LMM1215 × IIB386
IIB408	As IIB386, <i>recB1080 ΔrecQ::kan</i>	P1.LMM1215 × IIB388
IIB409	As IIB386, <i>recJ2052::Tn10kan</i>	P1.LMM1032 × IIB386
IIB410	As IIB386, <i>recB1080 recJ2052::Tn10kan</i>	P1.LMM1032 × IIB388
IIB420	As IIB386, <i>recB268::Tn10</i>	P1.N3071 × IIB386
IIB421	As IIB386, <i>recB268::Tn10 ΔrecQ::kan</i>	P1.LMM1215 × IIB420
IIB422	As IIB386, <i>recB268::Tn10 recO1504::Tn5</i>	P1.IRB103 × IIB420
IIB423	As IIB386, <i>recB268::Tn10 recJ2052::Tn10kan</i>	P1.LMM1032 × IIB420
IIB424	As IIB386, <i>recA269::Tn10</i>	P1.N3072 × IIB386
IIB570	As IIB386, <i>recR256::Tn10kan</i>	P1.AM208 × IIB386
IIB571	As IIB386, <i>recF400::Tn5</i>	P1.IIB294 × IIB386
IIB572	As IIB386, <i>recB1080 recR256::Tn10kan</i>	P1.AM208 × IIB388
IIB573	As IIB386, <i>recB1080 recF400::Tn5</i>	P1.IIB294 × IIB388
IIB574	As IIB386, <i>recB1080 recD1903::Tn10d(Tet) recR256::Tn10kan</i>	P1.AM208 × IIB390
IIB575	As IIB386, <i>recB1080 recD1903::Tn10d(Tet) recF400::Tn5</i>	P1.IIB294 × IIB390
IIB576	As IIB386, <i>recB268::Tn10 recR256::Tn10kan</i>	P1.AM208 × IIB420
IIB577	As IIB386, <i>recB268::Tn10 recF400::Tn5</i>	P1.IIB294 × IIB420

(*recB1080 recO* double mutant), there was almost no SOS induction and the IF was around 1 (Fig. 2C). This was consistent with the requirement of RecA loading activity for SOS induction after UV irradiation. In agreement with this, the *recB1080 recD recO* triple mutant showed SOS induction (IF, ~2.5) which was comparable to the SOS induction in *recO* (IF, ~3.5) and *recB1080* (IF, ~3.5) single mutants (Fig. 2C). Although the triple mutant exhibited a smaller effect on SOS induction, the SOS induction curve still differs from the SOS

induction curve for the *recB1080 recO* double mutant, in which there was almost no SOS induction (Fig. 2C). Similar results were obtained with *recB1080 recD recF* and *recB1080 recD recR* triple mutants (data not shown), as well as with the *recB1067* allele (another mutation in the gene encoding the nuclease center of RecB) (13) instead of *recB1080* (data not shown). All these data strongly suggest that RecA loading activity is essential for SOS induction.

We also looked for SOS induction in a *recB268 recO* double

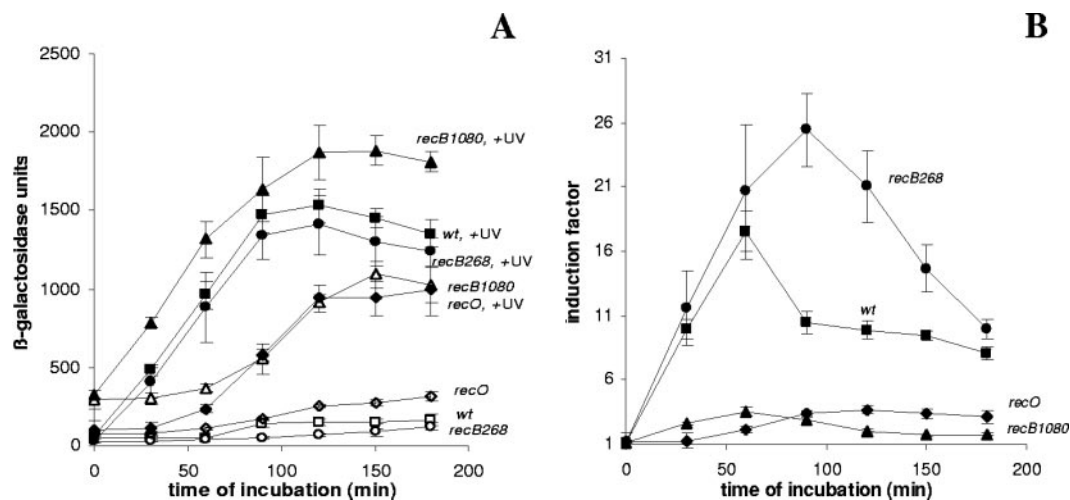


FIG. 1. (A) Induced levels of SOS expression (in β -galactosidase units) for the wt strain (strain IIB386) (■) and *recO* (strain IIB392) (◆), *recB1080* (strain IIB388) (▲), and *recB268* (strain IIB420) (●) mutants after UV irradiation during a 180-min incubation and basal levels of SOS expression (in β -galactosidase units) for the wt strain (strain IIB386) (□) and *recO* (strain IIB392) (◇), *recB1080* (strain IIB388) (△), and *recB268* (strain IIB420) (○) mutants during a 180-min incubation. (B) IFs (see Materials and Methods) for the wt strain (strain IIB386) (■) and *recO* (strain IIB392) (▲), *recB1080* (strain IIB388) (▲), and *recB268* (strain IIB420) (●) mutants after UV irradiation during a 180-min incubation. The symbols indicate means of at least three independent experiments, and the error bars indicate standard deviations.

mutant (Fig. 3). The *recB268* allele is a null allele which inactivates all of the functions of the RecBCD enzyme, including RecA loading ability. This double mutant was also deficient in both known mechanisms of RecA loading, and its SOS induction was much lower than that of the *recB268* single mutant (Fig. 3A) and wt cells (Fig. 1A). Unexpectedly, the low level of SOS induction was observed in this strain regardless of the inactivation of both known mechanisms of RecA loading. In Fig. 3C the data are expressed as IFs. Although the *recB268 recO* double mutant showed greater SOS induction (IF, ~ 3.7) than the *recB1080 recO* double mutant (IF, ~ 1) and the induction was comparable to the induction of the *recO* single mutant (IF, ~ 4), the absolute level of expression of β -galactosidase after UV irradiation in the *recB268 recO* double mutant was considerably lower than that in the *recB1080 recO* double mutant and that in the *recO* single mutant.

Effects of RecQ helicase and RecJ nuclease on SOS induction after UV irradiation. From the results described above, it is clear that RecA loading activity plays an important role in the SOS signaling mechanism after UV irradiation. We also wanted to test whether the RecQ helicase and the RecJ nuclease are important for SOS induction after UV irradiation. We compared SOS induction in the *recB1080* single mutant with SOS induction in the *recB1080 recQ* and *recB1080 recJ* double mutants (Fig. 4). In Fig. 4A the results are expressed in β -galactosidase units. The *recB1080 recQ* double mutant exhibited SOS induction after UV irradiation similar to that of the *recB1080* single mutant, implying that RecQ is not important for SOS induction in the *recB1080* background (Fig. 4A). When SOS induction was expressed as an IF, the level of SOS induction of the *recB1080 recQ* strain (IF ~ 2.5) was somewhat lower than the level of SOS induction of the *recB1080* single mutant (IF, ~ 3.5) (Fig. 4C). This can be explained by the fact that the *recB1080 recQ* double mutant had a slightly higher basal level of expression of the SOS response than the *recB1080* single mutant had (Fig. 4A). On the other hand, the *recJ* mutation moderately reduced SOS

induction in a *recB1080* background (Fig. 4B). The decrease in SOS induction was caused by the effect of the *recJ* mutation on the basal level of SOS expression in the *recB1080* background (compare the basal levels of SOS expression in the *recB1080* single mutant and the *recB1080 recJ* double mutant in Fig. 4B). This is discussed below (see Fig. 5). When the results were expressed as IFs, the *recB1080 recJ* double mutant had a higher level of SOS induction (IF, ~ 7) than the *recB1080* single mutant (Fig. 4C) because the *recB1080 recJ* double mutant had a much lower basal level of expression of the SOS response. These results support the conclusion that the RecQ helicase is not required for SOS induction after UV irradiation in a *recB1080* background and the conclusion that the RecJ nuclease is partially required due to its effect on basal SOS expression. We also examined the effects of *recQ* and *recJ* mutations in a *recB268* background (Fig. 3B). The *recB268 recQ* mutant had a somewhat higher level of SOS induction expressed in β -galactosidase units, whereas the *recB268 recJ* double mutant had a lower level of SOS induction than the *recB268* single mutant (Fig. 3B). Similar results were obtained when the data were expressed as IFs (Fig. 3C).

RecJ nuclease is required for a constitutive level of SOS expression in the *recB1080* mutant. We found that the *recB1080* mutant had a level of constitutive SOS expression response (about 1,100 β -galactosidase units) which was much higher than the levels of expression in the wt strain (~ 170 β -galactosidase units) and in *recO* (~ 300 β -galactosidase units), *recQ* (~ 200 β -galactosidase units), *recJ* (~ 200 β -galactosidase units), *recB268* (~ 150 β -galactosidase units), and *recA* (~ 80 β -galactosidase units) mutants (Fig. 1A and 5A). A comparison of the abilities of strains to achieve basal levels of SOS expression is summarized in Fig. 5B. The basal level of SOS expression in the *recB1080 recO* double mutant was a little lower but comparable to the basal level of SOS expression in the *recB1080* single mutant, implying that RecFOR-dependent RecA loading is not important for constitutive SOS expression in the *recB1080* mutant. Similar results were obtained with

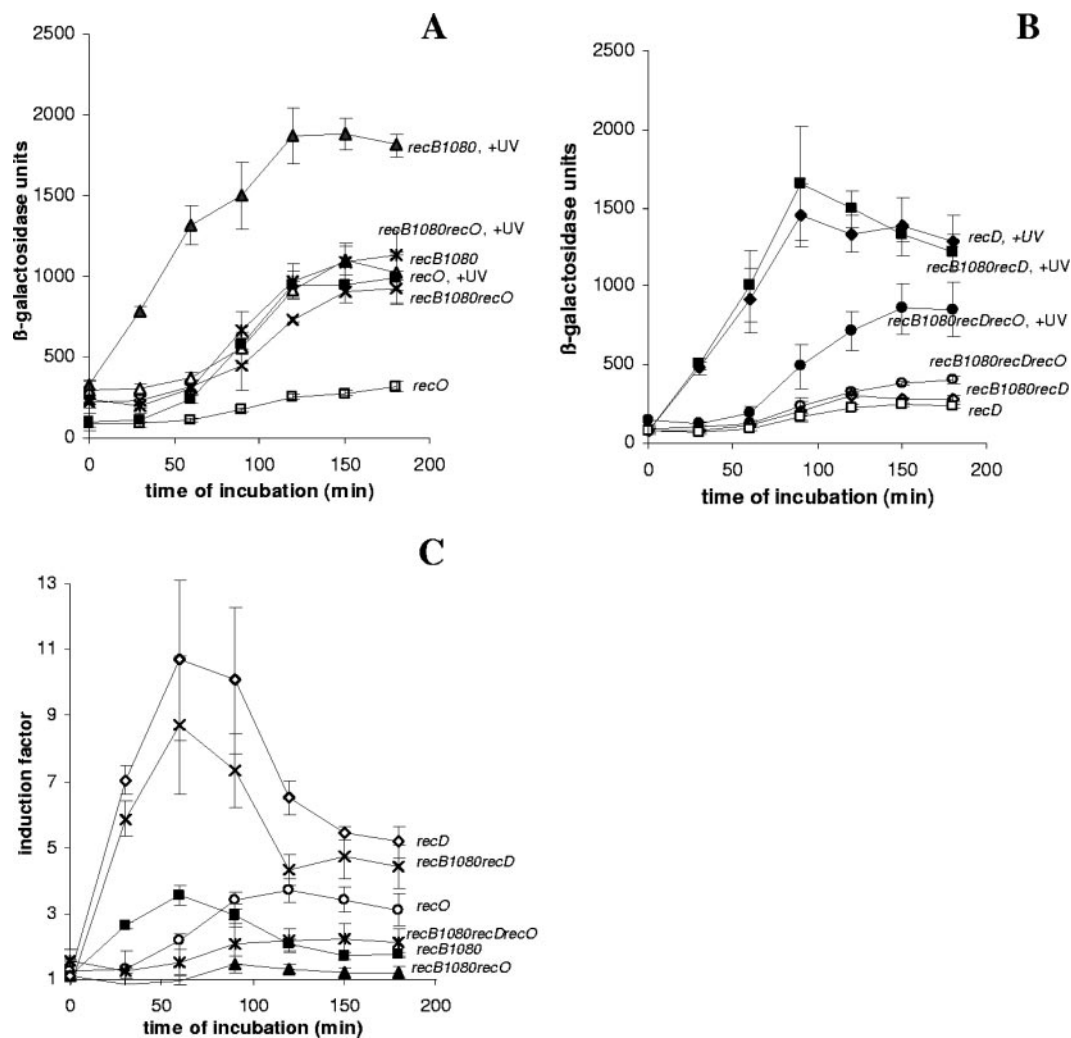


FIG. 2. (A) Induced levels of SOS expression (in β -galactosidase units) for *recB1080* (strain IIB388) (\blacktriangle), *recB1080 recO* (strain IIB401) ($*$), and *recO* (strain IIB392) (\blacksquare) mutants after UV irradiation during a 180-min incubation and basal levels of SOS expression (in β -galactosidase units) for *recB1080* (strain IIB388) (\blacktriangle), *recB1080 recO* (strain IIB401) (\times), and *recO* (strain IIB392) (\square) mutants during a 180-min incubation. (B) Induced levels of SOS expression (in β -galactosidase units) for *recD* (strain IIB395) (\blacksquare), *recB1080 recD* (strain IIB390) (\blacktriangle), and *recB1080 recD recO* (strain IIB393) (\bullet) mutants after UV irradiation during a 180-min incubation and basal levels of SOS expression (in β -galactosidase units) for *recD* (strain IIB395) (\square), *recB1080 recD* (strain IIB390) (\diamond), and *recB1080 recD recO* (strain IIB393) (\circ) mutants during a 180-min incubation. (C) IFs (see Materials and Methods) for *recO* (strain IIB392) (\circ), *recB1080* (strain IIB388) (\blacksquare), *recB1080 recO* (strain IIB401) (\blacktriangle), *recB1080 recD* (strain IIB390) (\times), *recB1080 recD recO* (strain IIB393) ($*$), and *recD* (strain IIB395) (\diamond) mutants after UV irradiation during a 180-min incubation. The symbols indicate means of at least three independent experiments, and the error bars indicate standard deviations.

recB1080 recF and *recB1080 recR* double mutants (data not shown). We also examined whether the RecJ nuclease and the RecQ helicase have any effect on the level of constitutive SOS expression in a *recB1080* mutant. The basal level of SOS expression in a *recB1080 recJ* double mutant (~ 350 β -galactosidase units) was considerably lower than the basal levels of SOS expression in *recB1080* single mutants ($\sim 1,100$ β -galactosidase units), implying that RecJ is required for constitutive SOS expression (Fig. 5B). In contrast, RecQ is not important for constitutive SOS expression since the constitutive SOS expression in the *recB1080 recQ* double mutant was comparable to the constitutive SOS expression in the *recB1080* single mutant (Fig. 5B). In addition, the level of constitutive SOS expression was also considerably lower in a *recB1080 recD* double mutant

(~ 300 β -galactosidase units), implying that the basal level of SOS expression in a *recB1080* strain is also *recD* dependent.

DISCUSSION

In this study we examined the effect of specific inactivation of RecA loading activity of the RecBCD enzyme on the SOS signaling mechanism. The effect of any mutation on SOS induction, expressed in β -galactosidase units, can be examined at two levels. One level is the effect on SOS induction due to DNA-damaging agents (external DNA damage, expressed by the IF), and the other level is the effect on basal SOS expression (internal DNA damage). For example, from our results it is obvious that the effects of *recFOR* mutations in a *recB1080*

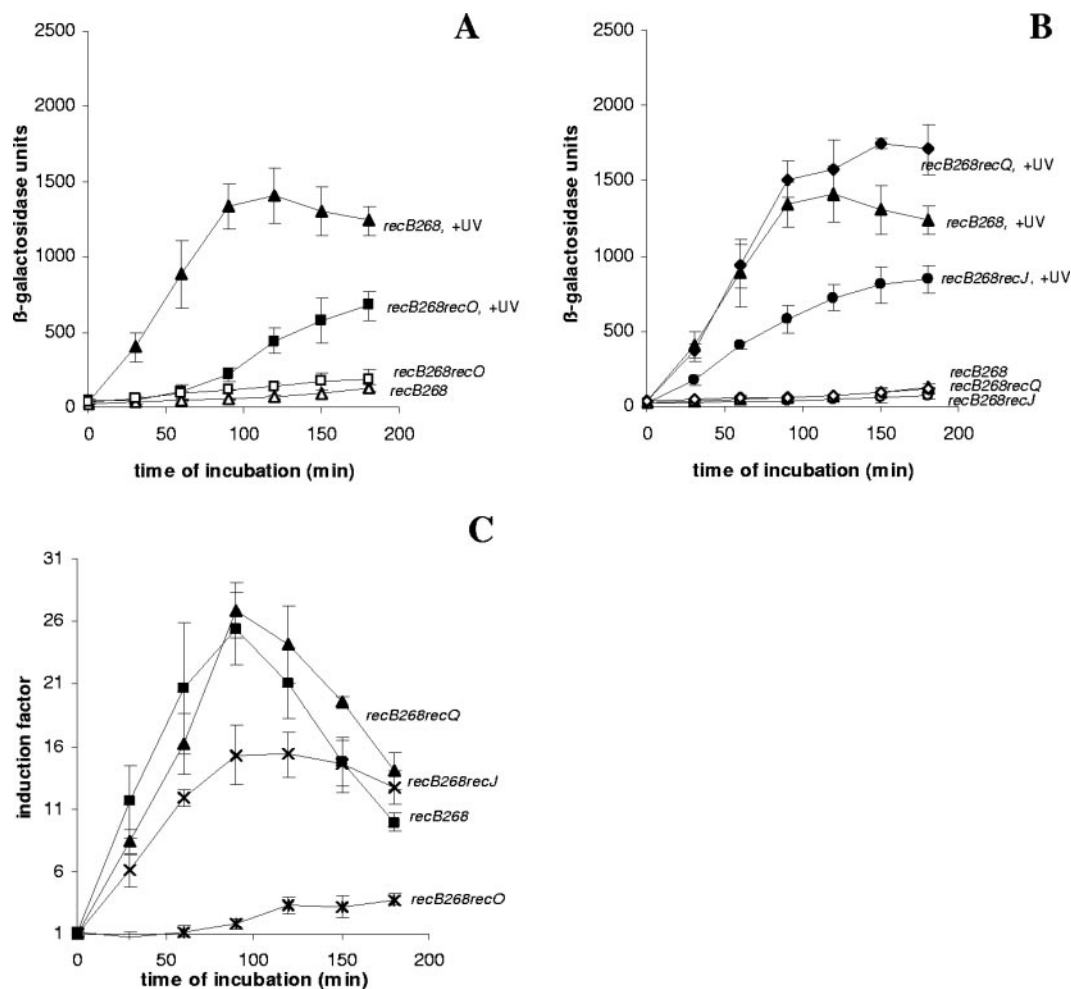


FIG. 3. (A) Induced levels of SOS expression (in β -galactosidase units) for *recB268* (strain IIB420) (▲) and *recB268 recO* (strain IIB422) (■) mutants after UV irradiation during a 180-min incubation and basal levels of SOS expression (in β -galactosidase units) for *recB268* (strain IIB420) (△) and *recB268 recO* (strain IIB422) (□) mutants during a 180-min incubation. (B) Induced levels of SOS expression (in β -galactosidase units) for *recB268 recQ* (strain IIB421) (◆), *recB268* (strain IIB420) (▲), and *recB268 recJ* (strain IIB423) (●) mutants after UV irradiation during a 180-min incubation and basal level of SOS expression (in β -galactosidase units) for *recB268 recQ* (strain IIB421) (◇), *recB268* (strain IIB420) (△), and *recB268 recJ* (strain IIB423) (○) mutants during a 180-min incubation. (C) IFs (see Materials and Methods) for *recB268* (strain IIB420) (■), *recB268 recO* (strain IIB422) (*), *recB268 recJ* (strain IIB423) (×), and *recB268 recQ* (strain IIB421) (▲) mutants after UV irradiation during a 180-min incubation. The symbols indicate means of at least three independent experiments, and the error bars indicate standard deviations.

background are at the level of SOS induction due to external agents (in this case UV irradiation) (Fig. 2), whereas the effect of a *recJ* mutation is at the level of basal SOS expression (Fig. 4 and 5). We show here that when both mechanisms of RecA-ssDNA filament formation were inactivated, SOS induction was drastically reduced (see the curves for the *recB1080 recO* and *recB268 recO* double mutants in Fig. 2 and 3) compared to the SOS induction in the controls (*recB1080*, *recB268*, and wt strains). When either the RecBCD or RecFOR pathway is intact, SOS induction occurs, but the kinetics are different (Fig. 1). These results strongly support the hypothesis that active RecA loading onto ssDNA is important as part of the SOS signaling mechanism caused by an external agent (UV irradiation).

The main DNA damage after UV irradiation is intrastrand cross-links, and pyrimidine dimers are the most abundant type of DNA damage (8). Since pyrimidine dimers are noncoding

lesions, DNA replication stops at the site of the DNA lesion and reinitiates downstream from the noncoding lesion (11). This results in production of an SSG to which the SSB protein binds, producing SSGs complexed with SSB (20). These complexes are the substrates for the RecFOR machinery which specifically replaces the SSB with RecA, producing the RecA-ssDNA filament on SSG (14, 20, 25). Then the RecA protein is activated and can catalyze the self-cleavage of the LexA repressor. Since SSGs are formed before the dsDNA ends, the SOS induction at an early time after UV irradiation is dependent on the RecFOR system (see the SOS induction in *recB1080* and *recB268* mutants shown Fig. 1, 2, and 3). In contrast, later, when dsDNA ends are formed as the result of replication fork collapse, SOS induction is dependent on the RecBCD enzyme (see the SOS induction in the *recO* mutant and in the *recB1080 recD recO* triple mutant shown Fig. 1 and 2). However, our results (see the presence of some SOS in-

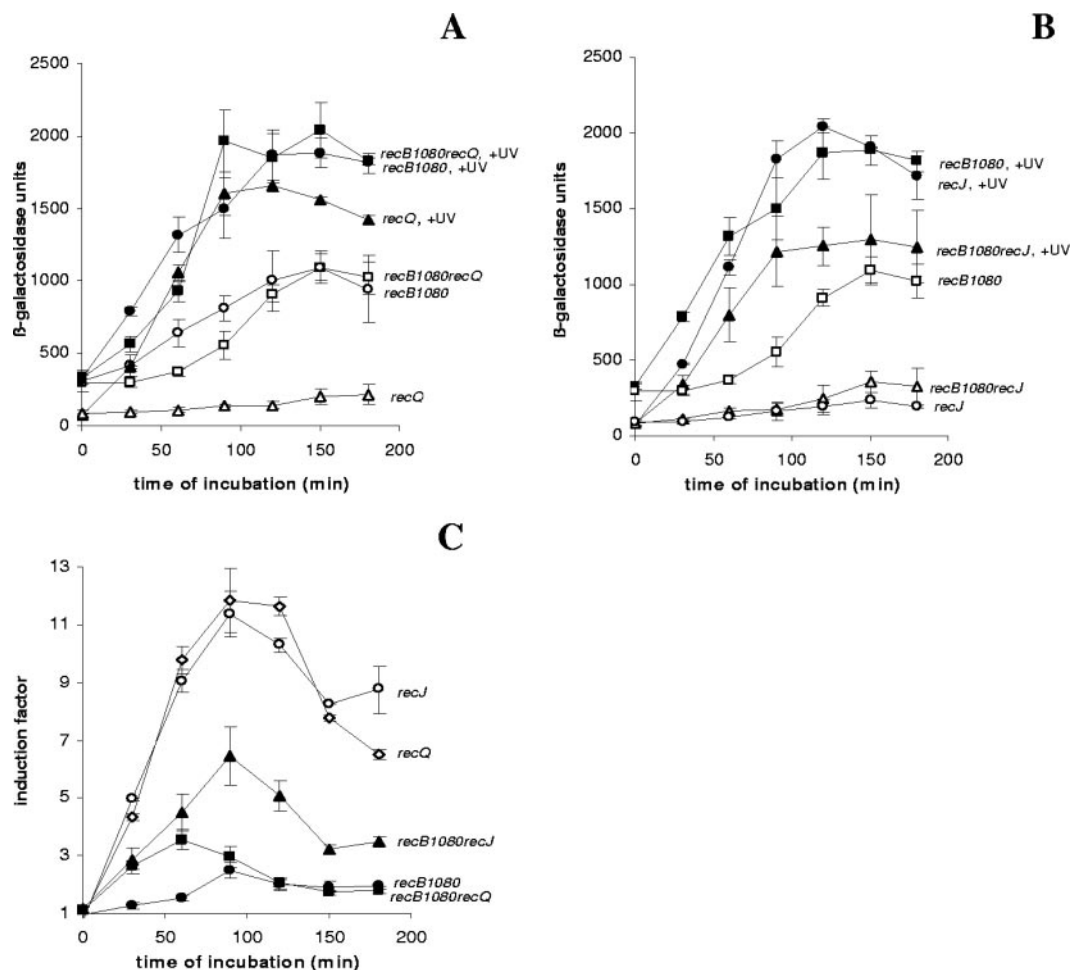


FIG. 4. (A) Induced levels of SOS expression (in β -galactosidase units) for *recB1080* (strain IIB388) (●), *recB1080 recQ* (strain IIB408) (■), and *recQ* (strain IIB407) (▲) mutants after UV irradiation during a 180-min incubation and basal levels of SOS expression (in β -galactosidase units) for *recB1080* (strain IIB388) (○), *recB1080 recQ* (strain IIB408) (□), and *recQ* (strain IIB407) (△) mutants during a 180-min incubation. (B) Induced levels of SOS expression (in β -galactosidase units) for *recB1080* (strain IIB388) (■), *recB1080 recJ* (strain IIB410) (▲), and *recJ* (strain IIB409) (●) mutants after UV irradiation during a 180-min incubation and basal levels of SOS expression (in β -galactosidase units) for *recB1080* (strain IIB388) (□), *recB1080 recJ* (strain IIB410) (△), and *recJ* (strain IIB409) (○) mutants during a 180-min incubation. (C) IFs (see Materials and Methods) for *recB1080* (strain IIB388) (■), *recB1080 recQ* (strain IIB408) (●), *recB1080 recJ* (strain IIB410) (▲), *recJ* (strain IIB409) (○), and *recQ* (strain IIB407) (◇) mutants after UV irradiation during a 180-min incubation. The symbols indicate means of at least three independent experiments, and the error bars indicate standard deviations.

duction in the *recB268 recO* double mutant shown in Fig. 3A and C) also suggest that some additional RecA loading mechanism (active or passive) could exist in the cell.

In the *recB1080* mutant recombination and dsDNA end repair are performed by a hybrid recombination mechanism in which helicase activity is provided by the RecB1080CD enzyme, 5'→3' exonuclease is provided by RecJ, and RecA loading is provided by RecFOR proteins (12). Our results show that the absence of RecJ nuclease has a moderate effect on SOS induction after UV irradiation in a *recB1080* genetic background (Fig. 4B). This effect is not as serious as the effect on recombination (12). Also, the absence of RecJ has a moderate effect on SOS induction in a *recB268* background (Fig. 3B). Our results also show that the RecQ helicase is not required for SOS induction in the *recB1080* (Fig. 4) and *recB268* backgrounds (Fig. 3). In *recB1080* and *recB268* mutants RecA loading activity is dependent on the RecFOR function. Obviously,

the most important substrates for SOS induction after UV irradiation are SSGs which are responsible for the high level of SOS induction before the appearance of dsDNA ends. Since SSGs are already regions of ssDNA, they do not need as much nuclease activity for the formation of ssDNA as is required for the processing of dsDNA ends. After UV irradiation, the RecJ nuclease together with the RecQ helicase could only extend the region of ssDNA within SSG, but these activities do not seem to be important for the further increase in SOS induction, as implied by our results. Possibly, the shorter RecA-ssDNA filaments are sufficient for SOS induction but not for recombination. This may be the explanation for the different genetic requirements for recombination and SOS induction with respect to a *recJ* mutation in a *recB1080* background.

In this study we also found that the *recB1080* mutant exhibited constitutive expression of the SOS regulon (Fig. 1A and 5). This phenotype is characteristic of some mutants that have

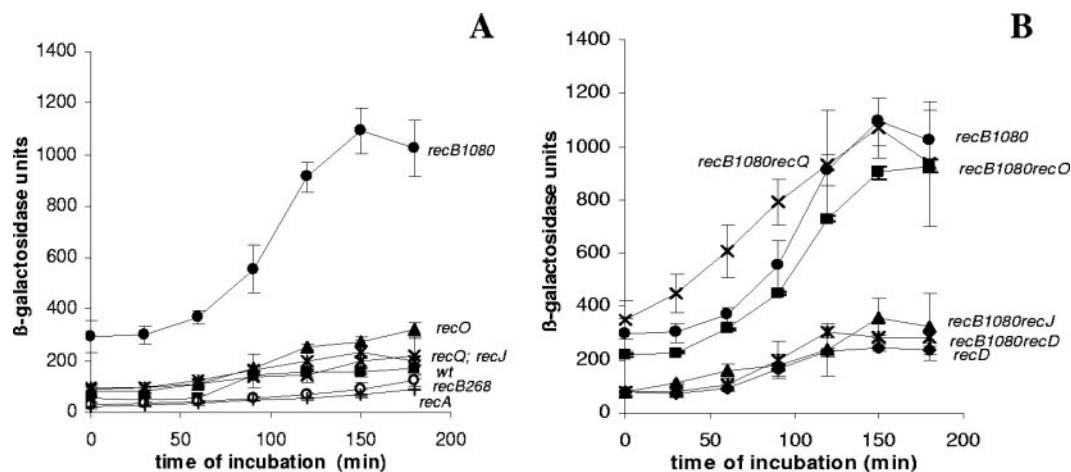


FIG. 5. (A) Basal levels of SOS expression (in β -galactosidase units) for *recB1080* (strain IIB388) (●), wt (strain IIB386) (■), *recO* (strain IIB392) (▲), *recQ* (strain IIB407) (×), *recJ* (strain IIB409) (*), *recB268* (strain IIB420) (○), and *recA* (strain IIB424) (+) strains during a 180-min incubation. (B) Basal levels of SOS expression (in β -galactosidase units) for *recB1080* (strain IIB388) (●), *recB1080 recO* (strain IIB401) (■), *recB1080 recJ* (strain IIB410) (▲), *recB1080 recQ* (strain IIB408) (×), *recD* (strain IIB395) (◆), and *recB1080 recD* (strain IIB390) (*) mutants during a 180-min incubation. The symbols indicate means of at least three independent experiments, and the error bars indicate standard deviations.

mutations in genes with defects in replication, recombination, and repair, including *rep*, *priA*, *dnaQ*, *recG*, *recN*, *dam*, *uvrD*, etc. (5, 6, 18, 19, 26, 28, 31, 32, 34). Constitutive SOS expression is caused by endogenous DNA damage which can lead to ssDNA and to replication fork collapse (19). The level of constitutive SOS expression (as well as the level of SOS induction) depends on two processes. One of these processes is the ability of the bacterial strain to obtain SOS expression by particular gene functions which are involved in the SOS signaling mechanism, and the other is the ability to repair DNA damage (time of persistence of DNA damage within the cell). The *recB1080* mutant is relatively resistant to UV irradiation, but it requires more time to complete repair, which is reflected in the lower viability of this mutant (12, 13). Consequently, in a *recB1080* mutant DNA lesions are likely to persist for a longer time, and this can explain the constitutive SOS expression in a *recB1080* mutant.

The genetic analysis of constitutive SOS expression in the *recB1080* mutant produced unexpected results. First, we found that RecFOR-mediated RecA-ssDNA filament formation is not important for constitutive SOS expression (see the constitutive SOS expression in a *recB1080 recO* double mutant shown Fig. 5). This result implies that some additional RecA loading mechanism independent of RecBCD and RecFOR could be present in *recB1080* cells. If the assumption that shorter RecA-ssDNA filaments are sufficient for SOS expression is correct, then this type of RecA loading could be a passive process. Second, we found that constitutive SOS expression in the *recB1080* mutant is dependent on the RecJ nuclease and that it is not present in a *recB1080 recD* double mutant (Fig. 5). This is the opposite of what we found for SOS induction due to an external agent (UV irradiation). It is possible that when the number of DNA lesions is small (basal level of endogenous DNA damage), the role of the RecJ nuclease and constitutive RecA loading activity independent of Chi (*recB1080 recD* double mutant) is more critical than the role after UV irradiation, when the number of lesions and SSGs is much higher. Perhaps

collapsed replication forks are the main endogenous DNA lesions, and the nuclease activities of RecJ and RecBCD could be important for the processing of these forks. An alternative explanation for the importance of RecJ in constitutive SOS expression is that RecJ is somehow involved in RecA loading independent of RecBCD and RecFOR (13).

Concluding remarks. In conclusion, we found that active RecA loading either by RecFOR or by RecBCD is necessary for SOS induction after UV irradiation. We also found that the absence of RecJ nuclease has a moderate effect on SOS induction in a *recB1080* mutant, whereas the RecQ helicase is not important. In addition, we found that RecJ (but not RecFOR and RecQ) is important for the constitutive SOS expression in a *recB1080* mutant, and this could be the explanation for the moderate effect of a *recJ* mutation on SOS induction caused by UV irradiation. The question which remains to be answered is the mechanism of RecA loading responsible for constitutive SOS induction in the *recB1080* mutant.

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