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sbcB15 and Δ*sbcB* Mutations Activate Two Types of RecF Recombination Pathways in *Escherichia coli*[▽]

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Escherichia coli **cells with mutations in *recBC* genes are defective for the main RecBCD pathway of recombination and have severe reductions in conjugational and transductional recombination, as well as in recombinational repair of double-stranded DNA breaks. This phenotype can be corrected by suppressor mutations in *sbcB* and *sbcC*(*D*) genes, which activate an alternative RecF pathway of recombination. It was previously suggested that *sbcB15* and Δ*sbcB* mutations, both of which inactivate exonuclease I, are equally efficient in suppressing the *recBC* phenotype. In the present work we reexamined the effects of *sbcB15* and Δ*sbcB* mutations on DNA repair after UV and γ irradiation, on conjugational recombination, and on the viability of *recBC* (*sbcC*) cells. We found that the *sbcB15* mutation is a stronger *recBC* suppressor than Δ*sbcB*, suggesting that some unspecified activity of the mutant SbcB15 protein may be favorable for recombination in the RecF pathway. We also showed that the *xonA2* mutation, a member of another class of ExoI mutations, had the same effect on recombination as Δ*sbcB*, suggesting that it is an *sbcB* null mutation. In addition, we demonstrated that recombination in a *recBC sbcB15 sbcC* mutant is less affected by *recF* and *recQ* mutations than recombination in *recBC* Δ*sbcB sbcC* and *recBC xonA2 sbcC* strains is, indicating that SbcB15 alleviates the requirement for the RecFOR complex and RecQ helicase in recombination processes. Our results suggest that two types of *sbcB*-sensitive RecF pathways can be distinguished in *E. coli*, one that is activated by the *sbcB15* mutation and one that is activated by *sbcB* null mutations. Possible roles of SbcB15 in recombination reactions in the RecF pathway are discussed.**

Homologous genetic recombination is a fundamental process that has two major roles in living cells: first, to facilitate DNA repair, thus maintaining chromosome integrity; and second, to rearrange genes within and between chromosomes, thereby promoting genetic diversity. The right balance between these two roles of recombination contributes considerably to cell survival and evolution.

In wild-type *Escherichia coli*, a number of recombination events proceed via the RecBCD-mediated pathway (13, 36). RecBCD is a complex multifunctional enzyme composed of three subunits encoded by the *recB*, *recC*, and *recD* genes. It recognizes blunt or nearly blunt double-stranded DNA (dsDNA) ends (39), which can arise in the cell by spontaneous or induced chromosome breakage or by DNA transfer during conjugation, transduction, and transformation. Starting from a dsDNA end, RecBCD initiates recombination by unwinding and simultaneously degrading DNA. Upon encountering a specific sequence designated Chi, the 3′-5′ nuclease activity of the enzyme is attenuated, and weaker 5′-3′ activity is activated (3). This nuclease modification allows production of a long 3′ single-stranded DNA (ssDNA) tail, onto which RecBCD directs loading of RecA protein (4, 5). A nucleoprotein filament created in this way plays a crucial role in further reactions of homologous DNA pairing and strand exchange.

recB and/or *recC* mutants of *E. coli* lack all RecBCD activities and exhibit severe recombination deficiency during genetic crosses, as well as sensitivity to various DNA-damaging agents (UV, ionizing radiation, chemical agents, etc.) that produce dsDNA breaks (12, 21). The low residual level of recombination in *recB*(*C*) null mutants can be restored to a level close to the wild-type level by extragenic *sbcB* and *sbcC*(*D*) suppressor mutations that activate an alternative RecF recombination pathway (named after *recF*, the first gene discovered in this pathway) (12, 19, 23, 25). Initiation of recombination in the RecF pathway depends on several enzymes that substitute for missing activities of RecBCD. Recombinogenic 3′ ssDNA overhangs are produced by the combined action of the RecQ helicase (or the UvrD and HelD helicases) and RecJ 5′-3′ ssDNA exonuclease, whereas the RecFOR proteins facilitate the loading of RecA protein onto prepared ssDNA (for reviews, see references 20 and 24).

The exact mechanism of activation of the RecF pathway by *sbc* mutations is not completely understood. Mutations in *sbcB* were shown to inactivate exonuclease I (ExoI), the enzyme that digests ssDNA in the 3′-5′ direction (23). It was assumed that elimination of ExoI activity by an *sbcB* mutation preserves recombinogenic 3′ ssDNA tails formed by the RecBCD-independent mechanism (12, 23). The *sbcC* and *sbcD* mutations accumulate spontaneously during propagation of *recBC sbcB* strains and are required for full suppression of the RecBC$^-$ phenotype (18, 25). The *sbcC* and *sbcD* genes belong to the same operon and code for subunits of the SbcCD nuclease (18). It was shown previously that SbcCD acts both as an endonuclease that cleaves hairpin structures and as an exonuclease that degrades linear dsDNA molecules (14, 15). Genetic

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evidence suggests that the SbcB and SbcCD enzymes have redundant roles in blunting UV-generated DNA ends prior to RecBC(D) action, implying that SbcCD has an exonuclease activity with 3′ protruding ends (35). Such an activity might explain the antirecombinogenic effect of the SbcCD enzyme in *recBC sbcB* cells and the necessity for *sbcC*(*D*) mutations in order to obtain full activation of the RecF pathway.

Most previous genetic studies of the RecF pathway were performed with *recBC sbcB sbcC*(*D*) strains carrying the *sbcB15* suppressor mutation. *sbcB15* belongs to the first group of *sbcB* mutations isolated, which were shown to suppress efficiently both DNA repair and the homologous recombination deficiency of *recBC* strains (23). These mutations were isolated after treatment of *recBC* cells with the chemical mutagen ethyl methane sulfonate (23), and they were mapped within the gene coding for ExoI (40). In further genetic characterization of the *sbcB* locus, two *sbcB* deletion mutants were also isolated and tested for *recBC* suppression in parallel with the *sbcB15* allele. It was found that the Δ*sbcB* and *sbcB15* mutations had the same suppressive effect on the RecBC$^-$ phenotype, increasing conjugational recombination and UV resistance to nearly wild-type levels (40). Concomitant with characterization of *sbcB* mutations, another class of ExoI mutations was identified after treatment of *recBC* cells with nitrosoguanidine. Interestingly, this class, designated *xonA* mutations, efficiently suppressed UV sensitivity but had only a modest suppressive effect on the conjugational recombination defect in *recBC* cells (22). The failure of *xonA* mutations to restore conjugational recombination with the same efficiency as *sbcB* was first suggested to result from a small amount of residual ExoI activity left in *xonA* cells (22). Later characterization of *xonA* mutations argued against this hypothesis since it was shown that some *xonA* mutants (*xonA2* and *xonA6*) are completely devoid of ExoI activity, whereas *sbcB15* mutants possess traces of this activity (31). Therefore, it was proposed that mutant ExoI in *xonA* strains might have some unspecified activity (other than ssDNA degradation) which interferes with RecF pathway enzymes (31).

Although early findings suggested that the *sbcB15* mutation was functionally equivalent to an *sbcB* deletion (and therefore could be considered a null mutation) (for a review, see reference 21), several later observations indicated that the two mutations had different effects on some recombination processes. A study of the role of ssDNA exonucleases in λ phage crosses revealed that the *sbcB15* allele inhibits the nucleolytic processing of DNA ends much more strongly than Δ*sbcB* inhibits this processing (32). Bidnenko et al. (9) studied recombinational repair in *rep* mutants (deficient for an auxiliary replicative helicase, the Rep protein), which suffer from frequent breakage of the replication fork and are therefore not viable in the absence of RecBCD. They found that Δ*sbcB sbcC* mutations restore the viability of *rep recBC* mutants, while *sbcB15 sbcC* mutations do not. Recently, the *sbcB15* mutation was found to increase the requirement for RuvABC proteins in recombinational repair after UV and γ irradiation, while an *sbcB* deletion had no such effect (43). Taking into account the finding that the *sbcB15* allele is efficiently expressed as a stable full-length product (31), it has been suggested that the mutant SbcB15 protein, although inactive as ExoI, might have some other activity (possibly DNA binding) that affects the recombination process (9, 32, 43).

In the present work we reexamined the effects of the *sbcB15*, Δ*sbcB*, and *xonA2* mutations on the RecBC$^-$ phenotype in different experimental systems by measuring DNA repair and recombination proficiency. The *sbcB* and *xonA* alleles were tested individually, as well as in combination with an *sbcC* mutation. We found that the effects of the two *sbcB* mutations differ significantly, and in the majority of tests the *sbcB15* mutation proved to be a stronger *recBC* suppressor than Δ*sbcB*. Our results also showed that the *xonA2* mutation has the same effect on recombination processes as Δ*sbcB*, suggesting that *xonA2* is a null mutation. In addition, we demonstrated that recombination in a *recBC sbcB15 sbcC* mutant shows an alleviated requirement for the RecFOR complex and RecQ helicase compared with *recBC* Δ*sbcB sbcC* and *recBC xonA2 sbcC* strains.

MATERIALS AND METHODS

Strains, media, and growth conditions. The *E. coli* strains used in this study are listed in Table 1. New strains were constructed by P1 transduction, as described by Miller (28). Transductants were isolated on LB medium plates (28) supplemented with appropriate antibiotics (chloramphenicol, 15 μg/ml; kanamycin, 50 μg/ml; tetracycline, 10 μg/ml) or on M9 plates (28) supplemented with glucose (0.4%), vitamin B1 (1 μg/ml), and all required amino acids (100 μg/ml). The phenotype of *sbcC201* transductants was confirmed by the increased efficiency of plating of λ phage carrying a 571-bp palindrome (18). λ*pal571* formed plaques on *sbcC* transductants with about 1,000-fold higher efficiency than it formed plaques on *sbcC*$^+$ strains. Transfer of the *xonA2* allele into the Δ*sbcB* recipient (Table 1) was additionally verified by PCR using primers 5′GACATG ATCTGTTGCCACTC3′ (upstream) and 5′CCATCACCGATTATCAGCAG3′ (downstream).

Bacterial cultures were grown in LB medium (28) at 37°C with shaking. Cell growth was monitored by measuring the optical density at 600 nm (OD_{600}). To determine the colony-forming ability of the strains, cells were appropriately diluted in phosphate buffer and plated on LB medium plates.

Irradiation experiments. Bacteria were grown from a single colony in LB medium at 37°C until the OD_{600} was 0.2. For UV irradiation experiments, serial dilutions of bacterial cultures were spotted on LB medium plates and irradiated with several doses of UV light (254 nm) at a dose of 0.5 J/m^2/s. The plates were incubated at 37°C for 24 to 48 h before the survivors were counted. In γ irradiation experiments, bacteria were pelleted by centrifugation, resuspended in cold phosphate buffer, and irradiated on ice with a ^{60}Co source at a dose rate of 12 Gy/s. Appropriate dilutions of irradiated cells were plated on LB agar, and colonies of survivors were scored after 24 to 48 h of incubation at 37°C.

Conjugational crosses. Hfr crosses were performed as described by Miller (28). Inheritance of the chromosomal Pro$^+$ marker was assayed. Donor (BW6156) and recipient strains were grown at 37°C to an OD_{600} of 0.3 before they were mixed at a ratio of 1:10. Mating was allowed to proceed for 25 min. *proAB*$^+$ recombinants were selected on M9 plates supplemented with glucose (0.4%), vitamin B1 (1 μg/ml), and all required amino acids (100 μg/ml) except proline. Streptomycin (100 μg/ml) was also added to the plates to counterselect donors.

RESULTS

Effects of *sbcB15* and Δ*sbcB* mutations on recombinational DNA repair in *recBC* (*sbcC*) cells. Early work on the RecF pathway was performed with strains initially considered to be *recBC sbcB* mutants. These strains were obtained by heavy mutagenic treatment of the *recB21 recC22* strain JC5519 (23), and one of them, JC7623 carrying the *sbcB15* mutation, is the strain that has been most widely used in further genetic studies. Later work by Lloyd and Buckman (25) revealed that JC7623 and some other *sbcB* derivatives of JC5519 had also acquired another suppressor mutation designated *sbcC*. To a certain

TABLE 1. *E. coli* strains

Strain[a]	Relevant genotype	Reference or source
AB1157	*rec*$^+$ *sbc*$^{+b}$	6
JC5519	*recB21 recC22*	6
JC7623	*recB21 recC22 sbcB15 sbcC201*	23
JC8260	*recB21 recC22 xonA2 hisG*$^+$	22
JJC260	*sbcD300*::*kan*	B. Michel
JJC889	Δ*sbcB*::*cam*	9
N2364	*sbcC201 phoR79*::Tn*10*	25
BW6156	Hfr *proAB*$^+$ (PO3 of P4X)	42
K797	*phoR79*::Tn*10*	CGSC 6456[c]
KL742	*hisG*$^+$ *thyA748*::Tn*10*	CGSC 6212[c]
V330	Δ(*recC-argA*)*234*	2
WA576	*recF400*::Tn*5*	B. Michel[d]
SWM1003	Δ*recQ*::*kan*	25
LMM981	*recB21 recC22 sbcB15 sbcC201 hisG*$^+$	P1.KL742 × JC7623 to His$^+$
LMM1124	*recB21 recC22* Δ*sbcB*::*cam*	P1.JJC889 × JC5519 to Cm^r
LMM1128	*recB21 recC22 sbcB15 hisG*$^+$	P1.LMM981 × LMM1124 to His$^+$ Cm^s UV^r
LMM1298	*recB21 recC22 sbcC201 phoR79*::Tn*10*	P1.N2364 × JC5519 to Tc^r λ*pal*^{s}
LMM1329	*recB21 recC22* Δ*sbcB*::*cam sbcC201 phoR79*::Tn*10*	P1.JJC889 × LMM1298 to Cm^r UV^r
LMM1330	*recB21 recC22 sbcB15 hisG*$^+$ *sbcC201 phoR79*::Tn*10*	P1.LMM981 × LMM1329 to His$^+$ Cm^s
LMM1331	*recB21 recC22* Δ*sbcB*::*cam sbcC201 phoR79*::Tn*10 recF400*::Tn*5*	P1.WA576 × LMM1329 to Km^r UV^s
LMM1332	*recB21 recC22 sbcB15 hisG*$^+$ *sbcC201 phoR79*::Tn*10 recF400*::Tn*5*	P1.WA576 × LMM1330 to Km^r UV^s
LMM1362	*recB21 recC22* Δ*sbcB*::*cam sbcC201 phoR79*::Tn*10* Δ*recQ*::*kan*	P1.SWM1003 × LMM1329 to Km^r UV^s
LMM1363	*recB21 recC22 sbcB15 hisG*$^+$ *sbcC201 phoR79*::Tn*10* Δ*recQ*::*kan*	P1.SWM1003 × LMM1330 to Km^r UV^s
LMM1745	*recB21 recC22 xonA2 hisG*$^+$ *sbcC201 phoR79*::Tn*10*	P1.JC8260 × LMM1329 to His$^+$ Cm^s
LMM1746	*recB21 recC22 xonA2 hisG*$^+$ *sbcC201 phoR79*::Tn*10 recF400*::Tn*5*	P1.WA576 × LMM1745 to Km^r UV^s
LMM1747	*recB21 recC22 xonA2 hisG*$^+$ *sbcC201 phoR79*::Tn*10* Δ*recQ*::*kan*	P1.SWM1003 × LMM1745 to Km^r UV^s
LMM1748	*recB21 recC22 xonA2 hisG*$^+$ *sbcD300*::*kan phoR*$^+$	P1.JJC260 × LMM1745 to Km^r Tc^s
LMM1749	*recB21 recC22 xonA2 hisG*$^+$ *sbcD*$^+$ *phoR79*::Tn*10*	P1.K797 × LMM1748 to Tc^r Km^s UV^s λ*pal*^{r}
LMM1721	*thyA748*::Tn*10*	P1.KL742 × AB1157 to Tc^r *thy*
LMM1724	Δ(*recC-argA*)*234 thyA*$^+$	P1.V330 × LMM1721 to *thy*$^+$ UV^s T42^s
LMM1725	Δ(*recC-argA*)*234 thyA*$^+$ Δ*sbcB*::*cam*	P1.JJC889 × LMM1724 to Cm^r
LMM1726	Δ(*recC-argA*)*234 thyA*$^+$ *sbcB15 hisG*$^+$	P1.LMM981 × LMM1725 to His$^+$ UV^r
LMM1732	Δ(*recC-argA*)*234 thyA*$^+$ Δ*sbcB*::*cam sbcC201 phoR79*::Tn*10*	P1.N2364 × LMM1725 to Tc^r UV^r λ*pal*^{s}
LMM1733	Δ(*recC-argA*)*234 thyA*$^+$ *sbcB15 hisG*$^+$ *sbcC201 phoR79*::Tn*10*	P1.LMM981 × LMM1732 to His$^+$ Cm^s

[a] All strains except BW6156, K797, KL742, and V330 are derivatives of AB1157.
[b] Other markers are F$^-$ *thr-1 ara-14 leuB6* Δ(*gpt-proA*)*62 lacY1 tsx-33 supE44 galK2* λ$^-$ rac$^-$ *hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1 qsr'*.
[c] Strain supplied by M. Berlyn of the *Escherichia coli* Genetic Stock Center.
[d] Strain originated from the laboratory of W. Wackernagel.

degree, this finding brought into question previous interpretations of results obtained with strains believed to be *recBC sbcB* strains, including the observation of Templin et al. (40) that *recBC sbcB15* and *recBC* Δ*sbcB* strains display equal recombination proficiencies.

To compare the effects of *sbcB15* and Δ*sbcB* mutations (individually or in combination with *sbcC*) on DNA recombination and recombinational repair, we introduced these mutations into the *recBC* strain JC5519 by P1 transduction (Table 1). It was previously demonstrated that *recBC sbcB15* mutants grow slowly and that fast-growing variants with mutations in *sbcC* or *sbcD* tend to accumulate spontaneously (18, 25). To avoid the possibility that uncharacterized suppressor mutations would influence our results, we carefully monitored the growth rates of our *recBC sbcB* constructs, frequently measuring the optical densities of exponential cultures and checking their typical small-colony phenotype. In addition, the SbcCD$^+$ phenotype of *recBC sbcB* mutants was confirmed by the low efficiency of plating of λ*pal571* phage.

The DNA repair proficiency of the mutants constructed was first tested after exposure to UV radiation (Fig. 1). As expected, the *recBC* mutant showed pronounced UV sensitivity; at the highest UV dose applied (40 J/m^2), its survival decreased almost 3 orders of magnitude compared to the survival of the wild-type strain (Fig. 1A). Both Δ*sbcB* and *sbcB15* mutations considerably improved the survival of *recBC* cells exposed to UV light. However, while the effect of Δ*sbcB* was only partial, (Δ*sbcB* increased the survival about 10-fold at a dose of 40 J/m^2), the *sbcB15* mutation showed stronger suppression, resulting in a level of repair proficiency much closer to the wild-type level (Fig. 1A).

In agreement with previous results (25), the *sbcC* mutation alone had no suppressive effect on the RecBC$^-$ phenotype (Fig. 1B). It also had no further effect on *recBC* UV sensitivity when it was combined with *sbcB15*. However, the *sbcC* mutation enhanced the suppressive effect of Δ*sbcB* so that the *recBC* Δ*sbcB sbcC* strains showed approximately the same UV resistance as *recBC sbcB15* and *recBC sbcB15 sbcC* strains (Fig. 1A and B). As the *recBC sbcB15* mutant behaved in UV repair exactly like the *recBC sbcB15 sbcC* mutant, we also examined whether there was a cryptic *sbcC* or *sbcD* suppressor mutation that was responsible for the high level of UV resistance of the former strain. Assuming that the presence of such a cryptic suppressor would also increase the survival of *recBC* Δ*sbcB* cells, we transduced the Δ*sbcB* mutation into the *recBC sbcB15* strain and tested the transductants to determine their repair

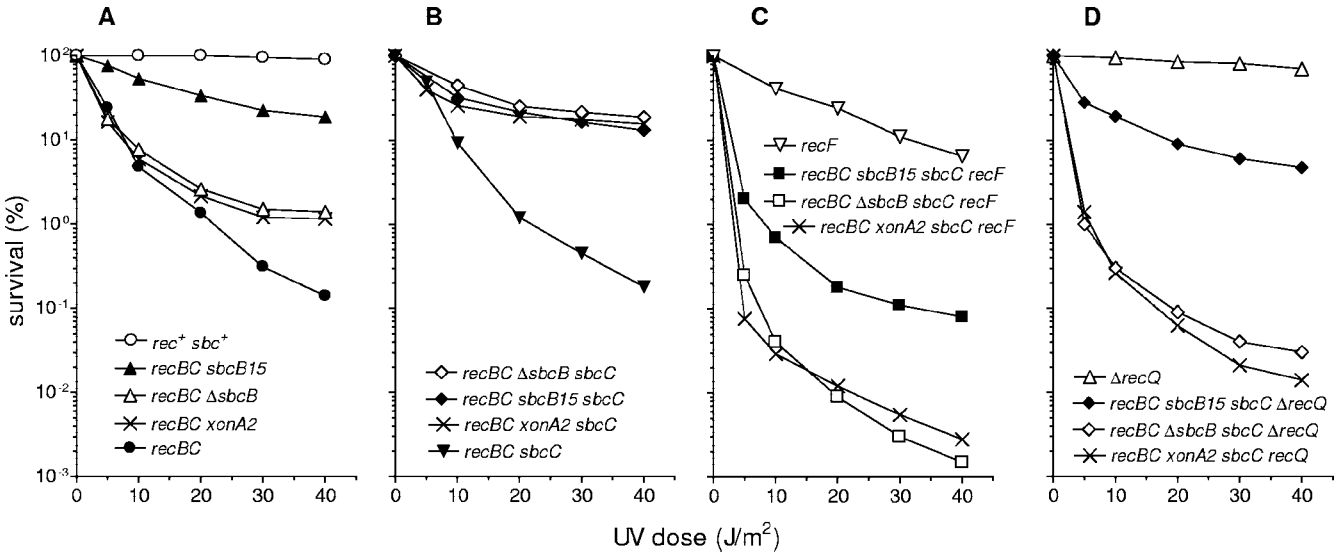

FIG. 1. Effects of *sbcB15*, Δ*sbcB*, and *xonA2* mutations on survival of different *recB21 recC22* derivatives exposed to UV irradiation. The strains used were AB1157 (*rec*$^+$ *sbc*$^+$), JC5519 (*recBC*), LMM1124 (*recBC* Δ*sbcB*), LMM1128 (*recBC sbcB15*), and LMM1749 (*recBC xonA2*) (A); LMM1298 (*recBC sbcC*), LMM1329 (*recBC* Δ*sbcB sbcC*), LMM1330 (*recBC sbcB15 sbcC*), and LMM1745 (*recBC xonA2 sbcC*) (B); WA576 (*recF*), LMM1331 (*recBC* Δ*sbcB sbcC recF*), LMM1332 (*recBC sbcB15 sbcC recF*), and LMM1746 (*recBC xonA2 sbcC recF*) (C); and SWM1003 (Δ*recQ*), LMM1362 (*recBC* Δ*sbcB sbcC* Δ*recQ*), LMM1363 (*recBC sbcB15 sbcC* Δ*recQ*), and LMM1747 (*recBC xonA2 sbcC recQ*) (D). The data for each strain are averages of results from at least three independent experiments.

efficiencies. The Δ*sbcB* derivatives of the *recBC sbcB15* strain showed decreased UV survival compared to the survival of the parental strain (not shown), just as previously observed with the *recBC* Δ*sbcB* mutant (Fig. 1A). The results described above suggest that the *sbcB15* mutation alone is sufficient for maximal induction of UV repair via the RecF pathway (consistent with results of Lloyd and Buckman [25]), whereas with the *sbcB* deletion an additional mutation in *sbcC* is required to obtain the same repair efficiency.

In a further analysis the strains described above were examined to determine their sensitivity to γ irradiation. Again, the Δ*sbcB* and *sbcB15* mutations showed a marked difference in suppressing *recBC* sensitivity (Fig. 2A). As observed in the UV experiment, although Δ*sbcB* significantly increased the survival of *recBC* cells, the effect was moderate compared with that of the *sbcB15* mutation, which almost completely restored resistance to γ irradiation.

The *sbcC* mutation had no significant effect on sensitivity to γ irradiation in either the *recBC* or *recBC sbcB15* background (Fig. 2B), but it considerably increased the survival of *recBC* Δ*sbcB* cells. However, unlike the results of the UV experiment, the *recBC* Δ*sbcB sbcC* cells were still more sensitive to γ irra-

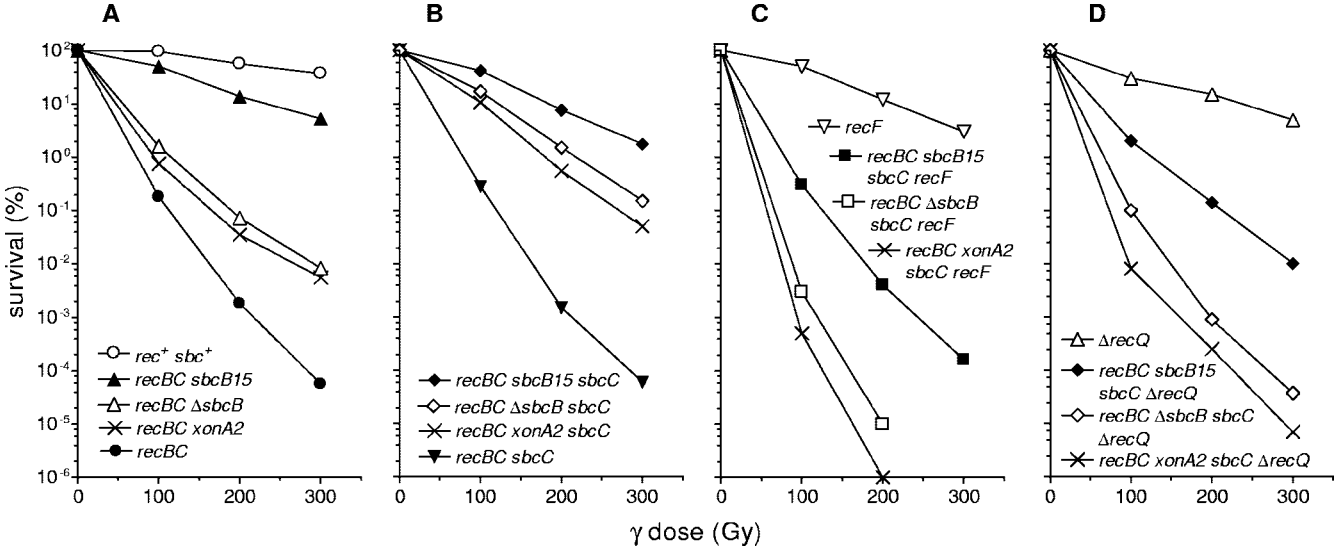

FIG. 2. Effects of *sbcB15*, Δ*sbcB*, and *xonA2* mutations on the γ irradiation sensitivity of different *recB21 recC22* derivatives. The strains used are listed in the legend to Fig. 1. The values are averages of results from at least three independent experiments.

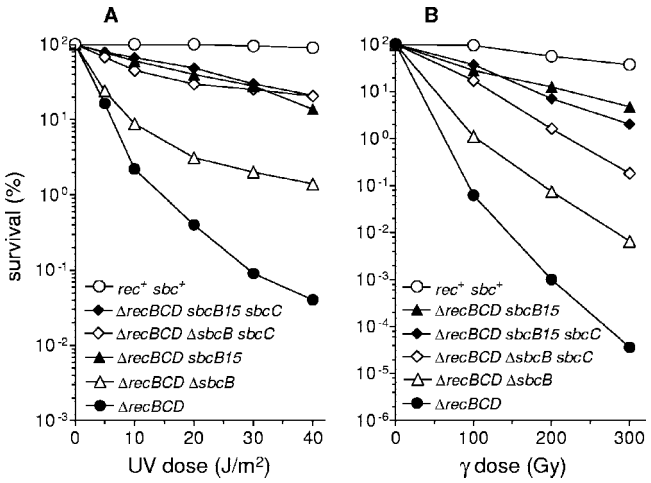

FIG. 3. Effects of *sbcB15* and Δ*sbcB* mutations on survival after UV and γ irradiation in a Δ*recBCD* background. The strains used were AB1157 (*rec*$^+$ *sbc*$^+$), LMM1724 (Δ*recBCD*), LMM1725 (Δ*recBCD* Δ*sbcB*), LMM1726 (Δ*recBCD sbcB15*), LMM1732 (Δ*recBCD* Δ*sbcB sbcC*), and LMM1733 (Δ*recBCD sbcB15 sbcC*). The values are averages of results from at least three independent experiments.

diation than either *recBC sbcB15* or *recBC sbcB15 sbcC* cells (Fig. 2A and B). Therefore, we concluded that after γ irradiation, the *sbcB15* mutation alone almost completely restores the repair proficiency of *recBC* cells, whereas the *sbcB* deletion cannot provide full suppression even when it is combined with *sbcC*.

The analysis of *sbcB* mutations described above was performed in a *recB21 recC22* background so that our results could be readily compared with previous analyses in which the workers predominantly used the same genetic background. The *recB21* and *recC22* mutations are classical mutations that are widely used in genetic studies, and according to all available data, either of these mutations is sufficient to abolish all known activities of the RecBCD enzyme (for reviews, see references 21 and 38). The *recB21* allele contains a 1.4-kb insertion (IS*186*) in its coding region and is polar on *recD* (1, 2), whereas *recC22* carries a UGA nonsense mutation (41) that presumably results in a truncated protein. To exclude the possibility that mutated RecB21 and RecC22 proteins influenced our results, the effects of *sbcB* mutations on recombinational repair were also examined with a strain having a complete deletion of the *recBCD* genes. We found that in a Δ*recBCD* background, the *sbcB* mutations behaved essentially in the same way that they behaved in *recB21 recC22* cells; i.e., *sbcB15* proved to be a stronger suppressor of the RecBCD$^-$ phenotype than Δ*sbcB* (Fig. 3A and B).

***sbcB15* and Δ*sbcB* mutations affect conjugational recombination differently in *recBC* (*sbcC*) mutants.** We further tested the recombination proficiency of different *sbcB* derivatives in conjugational crosses. During conjugation, ssDNA is transferred from the Hfr donor strain to the F$^-$ recipient, where it provides a template for DNA synthesis (for a review, see reference 17). When mating terminates, the transferred DNA is released as a double-stranded linear fragment that recombines with the recipient chromosome. This type of recombination can occur via either the RecBCD pathway (in wild-type cells) or the RecF pathway [in *recBC sbcBC*(*D*) mutants].

As shown in Table 2, in the *recBC* mutant the frequency of conjugational recombination decreased more than 100-fold, a result in accord with previous studies (16, 26). Introducing the Δ*sbcB* mutation into the *recBC* background had only a mild effect, increasing the frequency of recombination approximately twofold. The *sbcB15* mutation had a much stronger

TABLE 2. Conjugational recombination with different *sbcB* recipient strains

Recipient strain	Relevant genotype	Relative viability[a]	Relative yield of recombinants[b]
AB1157	Wild type	1	1
JC5519	*recB21 recC22*	0.27 ± 0.022	0.008 ± 0.0014
LMM1124	*recB21 recC22* Δ*sbcB*	0.25 ± 0.04	0.015 ± 0.0016
LMM1128	*recB21 recC22 sbcB15*	0.18 ± 0.042	0.18 ± 0.054
LMM1298	*recB21 recC22 sbcC201*	0.33 ± 0.037	0.005 ± 0.001
LMM1329	*recB21 recC22* Δ*sbcB sbcC201*	0.39 ± 0.088	0.16 ± 0.038
LMM1330	*recB21 recC22 sbcB15 sbcC201*	0.75 ± 0.125	1.4 ± 0.24
LMM1331	*recB21 recC22* Δ*sbcB sbcC201 recF400*	0.08 ± 0.022	0.0022 ± 0.0013
LMM1332	*recB21 recC22 sbcB15 sbcC201 recF400*	0.12 ± 0.045	0.1 ± 0.02
LMM1362	*recB21 recC22* Δ*sbcB sbcC201* Δ*recQ*	0.36 ± 0.042	0.007 ± 0.002
LMM1363	*recB21 recC22 sbcB15 sbcC201* Δ*recQ*	0.52 ± 0.086	0.087 ± 0.014
LMM1749	*recB21 recC22 xonA2*	0.21 ± 0.026	0.017 ± 0.004
LMM1745	*recB21 recC22 xonA2 sbcC201*	0.41 ± 0.012	0.14 ± 0.046
LMM1746	*recB21 recC22 xonA2 sbcC201 recF400*	0.15 ± 0.061	0.0017 ± 0.0005
LMM1747	*recB21 recC22 xonA2 sbcC201* Δ*recQ*::*kan*	0.40 ± 0.056	0.0075 ± 0.0007
LMM1724	Δ*recBCD*	0.29 ± 0.032	0.0046 ± 0.0006
LMM1725	Δ*recBCD* Δ*sbcB*	0.23 ± 0.061	0.0075 ± 0.0039
LMM1726	Δ*recBCD sbcB15*	0.19 ± 0.04	0.16 ± 0.026
LMM1732	Δ*recBCD* Δ*sbcB sbcC201*	0.42 ± 0.11	0.13 ± 0.026
LMM1733	Δ*recBCD sbcB15 sbcC201*	0.73 ± 0.035	1.1 ± 0.10

[a] Viability was determined for cultures grown to an OD_{600} of 0.3 and is expressed relative to the number of CFU per milliliter in cultures of control recipient strain AB1157, which averaged 1.5×10^8 CFU/ml. The values are averages ± standard deviations of results from at least three independent experiments.

[b] The yields of recombinants are relative to the yield of control strain AB1157 and were corrected for any deficiency in the viability of the recipient strain. The average yield for control strain AB1157 was 4.5×10^5 CFU per ml of the mating mixture. The values are averages ± standard deviations of results from at least three independent experiments.

effect, showing 10-fold-stronger *recBC* suppression than Δ*sbcB*. However, the *recBC sbcB15* mutant still displayed significantly lower recombination than the wild-type strain.

The *sbcC* mutation further improved the recombination proficiency of both *recBC sbcB* mutants. In *recBC* Δ*sbcB* cells it caused a 10-fold increase in recombination, whereas in *recBC sbcB15* cells it caused an 8-fold increase, resulting in a moderate hyper-*rec* phenotype (Table 2). Interestingly, the *recBC* Δ*sbcB sbcC* strain showed the same recombination frequency as the *recBC sbcB15* strain, indicating that the combined effect of Δ*sbcB* and *sbcC* is necessary to match the level of suppression of the single *sbcB15* mutation.

Taken together, the results described above suggest that the suppressive effects of the *sbcB15* and Δ*sbcB* mutations on conjugational recombination differ significantly in both *recBC* and *recBC sbcC* backgrounds. As observed in UV and γ irradiation experiments, the *sbcB15* mutation proved to be a stronger suppressor of the RecBC$^-$ phenotype than Δ*sbcB* was. These findings were corroborated by the experiments in which the effects of the two *sbcB* mutations on conjugational recombination were studied in the Δ*recBCD* background, the results of which showed the same pattern of suppression that was observed in *recB21 recC22* cells (Table 2).

***xonA2* mutation affects recombinational repair and conjugational recombination in *recBC* (*sbcC*) cells in the same way as Δ*sbcB*.** In addition to two *sbcB* mutations, we tested the effects of the *xonA2* mutation on DNA repair after UV and γ irradiation. We found that *xonA2* had almost the same effect on UV and γ irradiation repair as Δ*sbcB*, moderately improving the survival of irradiated *recBC* mutants and providing greater resistance when it was combined with *sbcC* (Fig. 1A and B and 2A and B).

In conjugational crosses, the *xonA2* mutation resulted in a negligible increase in recombination in *recBC* mutants, and the effect was quite similar to that of Δ*sbcB* (Table 2). The frequency of recombination was significantly increased after introduction of an additional *sbcC* mutation. The *recBC xonA2 sbcC* mutant recombined with the same efficiency as the *recBC* Δ*sbcB sbcC* strain, indicating again that there was a striking similarity between the *xonA2*- and Δ*sbcB*-associated phenotypes. Given the results described above, we concluded that in suppression of the RecBC$^-$ defects, the *xonA2* mutation has characteristics of an *sbcB* null mutation.

Effects of *sbcB15*, Δ*sbcB*, and *xonA2* mutations on the viability of *recBC* cells. Populations of exponentially growing *recB*, *recC*, or double-mutant cells contain large proportions (70 to 80%) of nonviable cells (11) (Table 2), suggesting that there are frequent spontaneous dsDNA breaks that cannot be repaired in the absence of the RecBCD enzyme (10). In contrast to *recB*(*C*) strains, the *sbcB15 sbcC* derivatives are highly viable, indicating that endogenous DNA damage can be efficiently repaired by the RecF pathway (25). However, it seems that the joint effects of *sbcB15* and *sbcC* mutations are critical for this type of repair since *recB*(*C*) *sbcB15* strains are no more viable than their *recB*(*C*) parents (25) (Table 2).

To our knowledge, the effects of an *sbcB* deletion on the viability of *E. coli recB*(*C*) cells have not been described previously. According to our data, the viability of *recBC* Δ*sbcB* cells is as low as that of *recBC* and *recBC sbcB15* mutants (Table 2). As observed previously with the *recBC sbcB15* strain (25), an additional *sbcC* mutation also improved the viability of *recBC* Δ*sbcB* cells. However, in the latter case the effect was quite modest; the viability of *recBC* Δ*sbcB sbcC* cells was about one-half that of the *recBC sbcB15 sbcC* strain. Interestingly, the viability of the *recBC* Δ*sbcB sbcC* strain was similar to that of *recBC sbcC* (Table 2), suggesting that the improvement in viability of the former strain was primarily due to the *sbcC* mutation rather than to Δ*sbcB*. We therefore concluded that the Δ*sbcB* mutation has no effect on viability in the *recBC* mutant and has only a modest effect in the *recBC sbcC* background.

We also measured the viability of *recBC xonA2* and *recBC xonA2 sbcC* strains. Briefly, the viability of *xonA2* derivatives was almost identical as that of their Δ*sbcB* counterparts, showing that *xonA*2 and Δ*sbcB* have the same effect on repair of spontaneously occurring DNA damage in *recBC* (*sbcC*) cells (Table 2).

Effects of *recF* and *recQ* mutations on recombination in *recBC sbcBC* and *recBC xonA sbcC* mutants. The results described above show that the *sbcB15* mutation produces a stronger suppressive effect in the *recBC* (*sbcC*) background than Δ*sbcB* produces. It is possible that this effect is due to some residual activity of the SbcB15 mutant protein, which could modify the enzymology of reactions in the RecF recombination pathway. To test this hypothesis, we examined the effect of a *recF* null mutation on recombination processes in *recBC sbcB15 sbcC* and *recBC* Δ*sbcB sbcC* strains. This mutation inactivates the RecF protein, a component of the RecFOR complex known to play an important role in the initiation of recombination in the RecF pathway (i.e., in the formation of the RecA nucleoprotein filament) (30).

After exposure to UV light, the *recF* mutation moderately decreased the survival of wild-type cells and had more pronounced effects in both the *recBC sbcB15 sbcC* and *recBC* Δ*sbcB sbcC* strains (Fig. 1C). However, the *recBC* Δ*sbcB sbcC recF* mutant proved to be much more UV sensitive than its *sbcB15* counterpart. The results obtained with γ-irradiated cells were quite similar to those obtained in UV experiments. Again, the *recF* mutation severely affected the survival of both *recBC sbcBC* strains, with a more severe effect in the Δ*sbcB* derivative (Fig. 2C). These results suggest that the *sbcB15* mutation alleviates the requirement for *recF* function during UV and γ irradiation repair in the RecF pathway.

The difference in the effects of the *recF* mutation on the recombination proficiency of *recBC sbcB15 sbcC* and *recBC* Δ*sbcB sbcC* strains was also observed in conjugational crosses. The decreases in recombination due to the *recF* mutation were about 10-fold in the *sbcB15* background and about 70-fold in the Δ*sbcB* background (Table 2). The collective results of the conjugational experiments suggest that the *recF* mutation completely nullifies suppression of the RecBC$^-$ phenotype by Δ*sbcB sbcC* mutations. In contrast, inactivation of the *recF* gene only partially impaired *recBC* suppression by *sbcB15 sbcC*.

The results obtained with *recF* mutants suggested that the SbcB15 protein influences the initial phase of the recombination process. To verify this suggestion, we tested the effect of a *recQ* mutation on recombination in the two *recBC sbcBC* backgrounds. The *recQ* mutation inactivates the principal DNA helicase of the RecF pathway, the RecQ protein, whose activity

is thought to substitute for the DNA unwinding activity of the RecBCD enzyme (21, 27).

In UV and γ irradiation experiments, the effect of a *recQ* mutation was quite similar to the effect of a *recF* mutation; i.e., inactivation of RecQ affected the repair more strongly in *recBC* Δ*sbcB sbcC* mutants than in *recBC sbcB15 sbcC* strains (Fig. 1D and 2D). Also, in conjugational crosses the *recQ* mutation reduced recombination in *recBC* Δ*sbcB sbcC* cells to the level of a *recBC* mutant, whereas in a *recBC sbcB15 sbcC* background it had a partial effect, allowing significant residual recombination to proceed (Table 2).

Since in all recombination assays performed the *xonA2* mutation showed the same phenotype as Δ*sbcB*, we wanted to examine whether the *recBC xonA2 sbcC* mutant exhibits a high requirement for RecF and RecQ proteins, like the *recBC* Δ*sbcB sbcC* strain. Indeed, we found that *recF* and *recQ* mutations severely affected both DNA repair (Fig. 1 and 2) and conjugational recombination (Table 2) in *recBC xonA2 sbcC* cells, similar to the effects observed with the *recBC* Δ*sbcB sbcC* mutant. These results complement our general finding that the effects of the *xonA2* mutation on recombination in *recBC* (*sbcC*) cells resemble those of an *sbcB* null mutation.

DISCUSSION

The results of this work show that the *sbcB15* and Δ*sbcB* mutations differ substantially in their abilities to suppress the recombinational deficiency of *E. coli recBC* mutants. In the majority of recombinational tests performed with *recBC sbcB* mutants (i.e., in assays of UV and γ irradiation repair and in conjugational crosses), *sbcB15* had a much stronger suppressive effect than Δ*sbcB*. Also, the *sbcB15* mutation suppressed the low-viability phenotype of *recBC* cells more strongly than Δ*sbcB* suppressed this phenotype, although in this case the suppressive effect of *sbcB* mutations was detectable only in the presence of an additional *sbcC* mutation. The results obtained clearly indicate that maximal *sbcB*(*C*)-dependent suppression of the RecBC$^-$ phenotype cannot be brought about by Δ*sbcB* (i.e., by complete abolition of ExoI). Instead, it seems that some residual activity of the mutant SbcB15 protein is favorable for recombination (at least in the experimental systems that we used) and is necessary for full suppression of the recombination defect in *recBC* cells.

Interestingly, a class of *sbcB* mutations resulting in a different phenotype than the *sbcB* deletion has also been isolated for *Salmonella enterica* serovar Typhimurium, a bacterium closely related to *E. coli* (8). One of these mutations, designated *sbcB1*, was thoroughly analyzed in several recombination tests and was demonstrated to be a stronger *recB* suppressor than Δ*sbcB*, suggesting that it could be a functional counterpart of the *E. coli sbcB15* mutation. The similarity of the results previously described for *S. enterica* serovar Typhimurium and those that we obtained with *E. coli* indicates that the two organisms have essentially the same mechanisms for regulating initiation of homologous recombination and repair in the RecF pathway.

The results of our study are, however, contrary to an old report suggesting that *sbcB15* and Δ*sbcB* have the same effect in restoring UV repair and conjugational recombination proficiency in *recB* mutants of *E. coli* (40). Using almost the same experimental conditions, we showed that the *recBC sbcB15* strain is considerably more proficient in recombination than the *recBC* Δ*sbcB* strain (Fig. 1A and Table 2). The discrepancy in the results could be partially explained if it is assumed that the *recB sbcB* strains used in the previous study carried in addition uncharacterized mutations in the *sbcC* and/or *sbcD* genes. According to our results obtained in UV irradiation experiments, an *sbcC* mutation abolishes the distinction between *recBC sbcB15* and *recBC* Δ*sbcB* strains, resulting in UV resistance close to that of the wild-type strain (Fig. 1). However, the same explanation cannot account for the discrepancy in conjugational crosses. Although we found that both *recBC sbcB15 sbcC* and *recBC* Δ*sbcB sbcC* strains recombine better than the *recBC* mutant, they still display a marked difference (almost 10-fold) in recombination frequency in favor of the *sbcB15* derivative (Table 2). A similar difference was also observed in conjugational crosses with newly constructed Δ*recBCD sbcB15 sbcC* and Δ*recBCD* Δ*sbc sbcC* derivatives of AB1157 (Table 2), as well as with the classical *recBC sbcB15 sbcC* strain JC7623 and its Δ*sbcB* derivative (not shown). Furthermore, this difference was also confirmed in transductional crosses involving the *recBC sbcBC* derivatives of strain MG1655 (not shown). A possible explanation for the high recombination proficiency of the *recB* Δ*sbcB* mutants used by Templin et al. could involve the way that these mutants were isolated; a deletion of the *sbcB* gene was constructed by P2 eduction, a method that leads to loss of 0.5 to 3 min of the *E. coli* chromosome (37, 40). We speculate that the part of the chromosome lost by P2 eduction might, in addition to *sbcB*, contain some other function interfering with recombination in the RecF pathway.

Besides the two *sbcB* mutations, we included a *xonA* mutation in our genetic analysis in an attempt to clarify the phenotypic similarities and differences previously reported to exist between the two classes of *recBC* suppressors (22). Although previous studies suggested that *xonA* mutations, like *sbcB* mutations, completely restore UV repair proficiency to *recBC* mutants, our results showed that the *xonA2* mutation only partially improves DNA repair, whereas full recovery requires the presence of an additional *sbcC* mutation (Fig. 1A and B). The simplest explanation for this difference in results was that the *recBC xonA* strains used in previous studies also contained uncharacterized *sbcC* or *sbcD* mutations. Indeed, when we plated λ*pal571* phage on the original *recBC xonA2* strain, strain JC8260, a high plating efficiency was obtained, revealing the *sbcC*(*D*) character of JC8260 (data not shown). Furthermore, our genetic analysis showed that in all experimental systems used, the *xonA2* mutation had the same effect on recombination and recombinational repair as Δ*sbcB* had. This observation, together with the previous finding that expression of the *xonA2* allele results in a truncated polypeptide completely devoid of nucleolytic activity (31), strongly suggests that *xonA2* might be an *sbcB* null mutation. Hence, our results do not support the hypothesis of Phillips et al. (31) that *xonA* mutations leave some residual nonnucleolytic ExoI activity which could hinder recombination. Rather, we assume that *xonA2* and other *xonA* mutants, like Δ*sbcB* cells, lack some feature that is present in *sbcB15* strains and stimulates recombinational processes.

In addition to our observation that the two types of *sbcB*

mutation have different suppressive effects on the RecBC$^-$ phenotype, we also found that they influence the enzymatic requirements of the RecF recombination pathway differently. The finding that the *recF* and *recQ* mutations more strongly affect recombination in *recBC* Δ*sbcB sbcC* and *recBC xonA2 sbcC* strains than in a *recBC sbcB15 sbcC* mutant suggests that the residual activity of the SbcB15 protein in the latter strain decreases the necessity for RecFOR and RecQ functions.

The possibility that SbcB15 might influence the enzymatic reactions in the RecF pathway was first proposed by Bidnenko et al. (9). These workers found that the Δ*sbcB* mutation facilitates the repair of broken replication forks in *rep recBC sbcCD* cells, whereas the *sbcB15* mutation has no beneficial effect on this type of repair. It was suggested that the SbcB15 protein obstructs DNA repair via the RecF pathway in the absence of a functional Rep helicase. After homologous pairing and D-loop formation, Rep helicase might be required to remove SbcB15 from the 3′ end, allowing recombination-dependent replication to occur (9). Hence, our results together with those of Bidnenko et al. indicate that the SbcB15 protein might modulate recombination reactions in the RecF pathway, decreasing the requirement for some proteins and increasing the requirement for others. In addition, these results suggest that (at least) two types of RecF pathway can be distinguished in *E. coli*, one that is activated by the *sbcB15* mutation and one that is activated by Δ*sbcB* (or other mutations that abolish all functions of ExoI).

What activity of the SbcB15 protein could account for stimulation of recombination via the RecF pathway? Previous genetic experiments indicated that in vivo the *sbcB15* mutation inhibits nucleolytic processing of 3′ ssDNA more strongly than Δ*sbcB* inhibits this processing (32). This finding led to the hypothesis that the mutant SbcB15 protein is able to bind 3′ ssDNA ends, thus preventing other nucleases from digesting the same substrate. Hence, the stronger inhibition of DNA degradation in the presence of SbcB15 might be due to a joint effect of ExoI inactivation and DNA protection (32). In the context of the RecF recombination pathway, such blocking of DNA ends by SbcB15 might preserve recombinogenic DNA ends better than the complete elimination of the SbcB protein preserves these ends (thus having a stronger suppressive effect on *recBC* mutations). If it is assumed that SbcB15 protects DNA ends from degradation by other exonucleases, it could be expected to interfere with the action of the SbcCD protein, a nuclease known to partially inhibit recombination in the RecF pathway (25). In this case, the stimulating effect of SbcCD inactivation on recombination should be significantly less pronounced in *recBC sbcB15* cells than in *recBC* Δ*sbcB* cells. Interestingly, our results obtained in UV and γ irradiation experiments fit this end protection model well, showing that the recombinational repair in *recBC sbcB15* mutants is much more resistant to the SbcCD nuclease than the recombinational repair in *recBC* Δ*sbcB* cells is (Fig. 1 and 2). In fact, after exposure to UV and γ radiation, the *recBC sbcB15* mutant was almost fully repair proficient so that inactivation of SbcCD nuclease by the *sbcC* mutation had little (if any) additional effect (Fig. 1 and 2). A quite different situation was observed with the *recBC* Δ*sbcB* strain, whose low repair proficiency was strongly improved by inactivation of *sbcC*.

Unlike the results of the irradiation experiments, in conjugational crosses the *sbcC* mutation significantly improved recombination in both *recBC sbcB15* and *recBC* Δ*sbcB* mutants, and the net increases in recombination frequency due to SbcCD inactivation were about equal (approximately 10-fold) in the two backgrounds (Table 2). This finding suggests that during conjugational recombination the SbcB15 protein cannot efficiently prevent the activity of the SbcCD enzyme and that the beneficial effect of SbcB15 on this type of recombination must be attributed primarily to some other mechanism.

The cell viability measurements are also difficult to accommodate with the end protection model discussed above. These measurements show that neither the *sbcB15* mutation nor the Δ*sbcB* mutation alone has any suppressive effect on the low-viability phenotype of exponentially growing *recBC* cells (Table 2). Only after additional inactivation of the *sbcC* gene was a strong increase in viability observed in *recBC sbcB15* cells, whereas in *recBC* Δ*sbcB* cells the *sbcC* mutation caused only a slight improvement in viability. These results clearly indicate that SbcB15 cannot prevent the antirecombinogenic action of SbcCD during repair of endogenous DNA damage.

Although the results of conjugational crosses and cell viability measurements are not readily explained by the end protection model, they do not exclude the possibility that in the absence of SbcCD activity, SbcB15 protects DNA from other 3′-5′ exonucleases which might antagonize the recombination process. This hypothesis is suggested by the fact that *recBC sbcB15 sbcC* mutants are far more viable and show higher proficiency in conjugational crosses than *recBC* Δ*sbcB sbcC* cells. Theoretically, the interplay between SbcB15 and SbcCD (and possibly other nucleases) could be influenced by the shape of the DNA ends exposed (e.g., by the presence of single-stranded overhangs that are different lengths and have different polarities, by chemical modifications of terminal deoxynucleoside triphosphates, etc.) and by the affinity of different nucleases for a particular end type. Hence, the variety of suppression patterns observed in our experiments with *recBC sbcB*(*C*) strains might reflect different DNA substrates present, leaving the possibility that at least in some cases SbcB15 has DNA-protecting activity. To address this question in more detail, additional experiments involving new *recBC sbcB*(*C*) derivatives in which residual 3′-5′ Exo activities are depleted are needed.

An alternative explanation for the prorecombinogenic activity of SbcB15 (that does not necessarily exclude the model described above) may involve a more active role of the mutant protein in the recombination process. This possibility is derived from in vitro studies suggesting that ExoI physically interacts with two proteins that have an important role in recombination, the SSB and RecA proteins (7, 34). The interaction between ExoI and SSB may be functionally important since it was shown that SSB stimulates the deoxyribophosphodiesterase activity of ExoI during the repair of abasic sites in DNA (33), as well as its 3′-5′ exonuclease activity with an ssDNA substrate (29). On the other hand, the possible relevance of an ExoI-RecA interaction remains to be elucidated. If SbcB15 retains the interacting properties of the wild-type enzyme (or if these properties are modified due to the mutation), it could conceivably influence the recombination process at the level of RecA filament formation. It has been demonstrated in vitro that the SSB and RecA proteins compete for the same substrate

(ssDNA) and that RecBCD or RecFOR activities are required to facilitate efficient loading of the RecA protein in the presence of SSB (4, 30). Since SbcB15 presumably favors recombination in *recBC* (*sbcC*) mutants, we speculate that it stimulates binding of RecA to ssDNA by dislodging the molecules of SSB protein. Such an activity could enhance the formation of RecA filaments, as well as the pairing of the filaments with homologous DNA, thus protecting DNA from nucleolytic degradation and increasing the overall efficiency of the recombination process. Our finding that the *sbcB15* mutation alleviates the requirement for RecFOR activity in the RecF pathway is in accord with this hypothesis. On the other hand, the relaxed requirement for RecFOR could also result from passive DNA end protection by SbcB15, which might provide enough time for RecA filaments to be made even under restrictive conditions (i.e., in the absence of RecFOR-mediated loading). In Δ*sbcB* mutants deficient for RecFOR function, DNA ends would be degraded by nucleases before they are engaged in RecA filament formation and homologous pairing.

It was previously shown that in the absence of RecQ helicase, the residual DNA unwinding activity provided by UvrD (helicase II) and HelD (helicase IV) allows recombination in the RecF pathway to proceed, although it proceeds with lower efficiency (27). Our finding that the *recQ* mutation more strongly affects recombination in a *recBC* Δ*sbcB sbcC* strain than in a *recBC sbcB15 sbcC* background suggests that SbcB15 alleviates the requirement for DNA unwinding activity during initiation of recombination. We hypothesize that when DNA ends are protected by SbcB15, even reduced DNA unwinding is sufficient to ensure substantial recombination. However, in *recBC* Δ*sbcB sbcC* cells, in which DNA ends are not protected, vigorous DNA unwinding is necessary to overcome residual 3′-5′ exonucleolytic activity in order to generate recombinogenic ssDNA tails. Experiments to test the hypotheses described above and to further elucidate the role of SbcB15 in recombination are under way in our laboratory.

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