The RuvABC Resolvase Is Indispensable for Recombinational Repair in *sbcB15* Mutants of *Escherichia coli*

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The RuvABC proteins of *Escherichia coli* play an important role in the processing of Holliday junctions during homologous recombination and recombinational repair. Mutations in the *ruv* genes have a moderate effect on recombination and repair in wild-type strains but confer pronounced recombination deficiency and extreme sensitivity to DNA-damaging agents in a *recBC sbcBC* background. Genetic analysis presented in this work revealed that the Δ*ruvABC* mutation causes an identical DNA repair defect in UV-irradiated *recBC sbcBC*, *sbcBC*, and *sbcB* strains, indicating that the *sbcB* mutation alone is responsible for the extreme UV sensitivity of *recBC sbcBC ruv* derivatives. In experiments with gamma irradiation and in conjugational crosses, however, *sbcBC* Δ*ruvABC* and *sbcB* Δ*ruvABC* mutants displayed higher recombination proficiency than the *recBC sbcBC* Δ*ruvABC* strain. The frequency of conjugational recombination observed with the *sbcB* Δ*ruvABC* strain was quite similar to that of the Δ*ruvABC* single mutant, indicating that the *sbcB* mutation does not increase the requirement for RuvABC in a recombinational process starting from preexisting DNA ends. The differences between the results obtained in three experimental systems used suggest that in UV-irradiated cells, the RuvABC complex might act in an early stage of recombinational repair. The results of this work are discussed in the context of recent recombination models which propose the participation of RuvABC proteins in the processing of Holliday junctions made from stalled replication forks. We suggest that the mutant SbcB protein stabilizes these junctions and makes their processing highly dependent on RuvABC resolvase.

Homologous recombination is a fundamental cellular process that promotes genetic diversity and plays an important role in repairing various types of DNA damage. In addition, homologous recombination participates in DNA replication by promoting the restoration of collapsed replication forks (22, 26, 27, 35).

In wild-type *Escherichia coli*, most recombination events are initiated by the RecBCD enzyme (10, 30, 44). The RecBCD pathway is essential for repair of double-stranded DNA breaks; it also participates in recombination following conjugation, transduction, and transformation as well as in vegetative phage crosses. The RecBCD enzyme initiates recombination by unwinding and simultaneously degrading DNA from a double-stranded DNA end. Upon encountering a specific sequence named Chi, the 3'-5' nuclease activity of the enzyme is attenuated, and a weaker nuclease activity of the opposite polarity, 5'-3', is activated (1, 14). This nuclease modification allows the production of a long 3'-terminal single-stranded DNA (ssDNA) tail, which is used by RecA protein to invade a homologous duplex DNA molecule (2, 3, 13).

Strains lacking *recBC* function are recombination deficient and sensitive to UV light, ionizing radiation, and other DNA-damaging agents (9). The residual level of recombination in *recBC* null mutants can be restored to the wild-type level by extragenic suppressor mutations *sbcB* and *sbcC(D)*. The *sbcB* mutations inactivate exonuclease I (ExoI), which digests ssDNA in the 3'-5' direction (24). It is presumed that elimi-

nation of ExoI by an *sbcB* mutation might prolong the life of ssDNA molecules as potential substrates for recombination. The *sbcC* mutation is needed for full suppression of *recBC* and accumulates spontaneously during the growth of *recBC sbcB* cells (29).

The same effect was observed with mutations in another gene, *sbcD*, located immediately downstream of *sbcC* (17). It was recently shown that the SbcCD complex acts as a nuclease that cleaves hairpin structures formed at replication forks (12). In *recBC sbcBC(D)* mutants, recombination proceeds via the RecF pathway, which requires the products of several genes, including *recQ*, *recJ*, *recF*, *recO*, and *recR* (reviewed in references 11 and 30). The joint action of the RecQ helicase and the RecJ nuclease on a DNA duplex provides the recombinogenic 3'-ssDNA which is a substrate for the RecA protein. The RecF, RecO, and RecR proteins facilitate the loading of RecA onto the ssDNA.

Homologous pairing and strand exchange reactions catalyzed by RecA lead to the formation of a Holliday junction, a recombination intermediate that consists of two homologous DNA duplexes linked by a single-stranded crossover. During the final stage of recombination, Holliday junctions are processed into mature recombinant molecules. Three Ruv proteins involved in this process, RuvA, RuvB, and RuvC, have been characterized (49). RuvA specifically recognizes Holliday junctions and allows the RuvB helicase to bind. Together, RuvA and RuvB catalyze branch migration of recombination intermediates, leading to the extension of heteroduplex DNA (21, 45). Additional binding of the RuvC endonuclease allows the RuvABC complex to resolve Holliday junctions by nicking strands of like polarity (5, 20).

Cells carrying mutations in any of the *ruv* genes display

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TABLE 1. *E. coli* strains

Strain	Relevant genotype	Source or reference
AB1157	Wild type	4
LMM10	Δ*ruvABC*::*cam*	P1.JJC768 × AB1157 to Cm^r UV^s
JC5519	*recB21 recC22*	4
LMM20	*recB21 recC22* Δ*ruvABC*::*cam*	P1.JJC768 × JC5519 to Cm^r
JC7623	*recB21 recC22 sbcB15 sbcC201*	4
LMM864	*recB21 recC22 sbcB15 sbcC201* Δ*ruvABC*::*cam*	P1.JJC768 × JC7623 to Cm^r UV^s
TNM734	*recB21 recC22 sbcB15 sbcC201 rus-1*	33
LMM867	*recB21 recC22 sbcB15 sbcC201 rus-1* Δ*ruvABC*::*cam*	P1.JJC768 × TNM734 to Cm^r
LMM963	*recB21 recC266*::Tn*10 sbcB15 sbcC201*	P1.N2103 × JC7623 to Tc^r
LMM964	*recB21 recC266*::Tn*10 sbcB15 sbcC201 rus-1*	P1.N2103 × TNM734 to Tc^r
LMM965	*fucP3154*::Tn*10 kan recB$^+$ recC$^+$ sbcB15 sbcC201*	P1.CAG12115 × LMM963 to Km^r Tc^s T42^r
LMM966	*fucP3154*::Tn*10 kan recB$^+$ recC$^+$ sbcB15 sbcC201* Δ*ruvABC*::*cam*	P1.JJC768 × LMM965 to Cm^r UV^s
LMM967	*fucP3154*::Tn*10 kan recB$^+$ recC$^+$ sbcB15 sbcC201 rus-1*	P1.CAG12115 × LMM964 to Km^r Tc^s T42^r
LMM969	*fucP3154*::Tn*10 kan recB$^+$ recC$^+$ sbcB15 sbcC201 rus-1* Δ*ruvABC*::*cam*	P1.JJC768 × LMM967 to Cm^r
LMM979	*fucP3154*::Tn*10 kan recB$^+$ recC$^+$ sbcB15 sbcC$^+$ phoR79*::Tn*10*	P1.K797 × LMM965 to Tc^r λ*pal^r*
LMM983	*fucP3154*::Tn*10 kan recB$^+$ recC$^+$ sbcB15 sbcC$^+$ phoR79*::Tn*10* Δ*ruvABC*::*cam*	P1.JJC768 × LMM979 to Cm^r UV^s
LMM984	*fucP3154*::Tn*10 kan recB$^+$ recC$^+$ sbcB15 sbcC$^+$ phoR79*::Tn*10 rus-1*	P1.K797 × LMM967 to Tc^r λ*pal^r*
LMM986	*fucP3154*::Tn*10 kan recB$^+$ recC$^+$ sbcB15 sbcC$^+$ phoR79*::Tn*10 rus-1* Δ*ruvABC*::*cam*	P1.JJC768 × LMM984 to Cm^r
N2364	*sbcC201*	29
LMM971	*sbcC201* Δ*ruvABC*::*cam*	P1.JJC768 × N2364 to Cm^r UV^s
LMM995	*recB21 recC22 sbcB15 sbcC201 zef-3129*::Tn*10*	P1.CAG12099 × JC7623 to Tc^r
LMM997	Δ*xonA300*::*cat*	P1.STL2694 × AB1157 to Cm^r
LMM1001	*sbcB15 zef-3129*::Tn*10*	P1.LMM995 × LMM997 to Tc^r Cm^s
LMM1005	*sbcB15 zef-3129*::Tn*10* Δ*ruvABC*::*cam*	P1.JJC768 × LMM1001 to Cm^r UV^s
LMM1015	*ruvB71*::*kan*	P1.N4680 × AB1157 to Km^r UV^s
LMM1017	Δ*xonA300*::*cat ruvB71*::*kan*	P1.N4680 × LMM997 to Km^r UV^s
LMM1018	*sbcB15 zef-3129*::Tn*10 ruvB71*::*kan*	P1.N4680 × LMM1001 to Km^r UV^s
LMM1032	*recJ2052*::Tn*10 kan*	P1.STL113 × AB1157 to Km^r
LMM1033	*recB21 recC22 sbcB15 sbcC201 recJ2052*::Tn*10 kan*	P1.STL113 × JC7623 to Km^r UV^s
LMM1034	*sbcB15 zef-3129*::Tn*10 recJ2052*::Tn*10 kan*	P1.STL113 × LMM1001 to Km^r
LMM1035	*sbcB15 zef-3129*::Tn*10 recJ2052*::Tn*10 kan* Δ*ruvABC*::*cam*	P1.STL113 × LMM1005 to Km^r
JJC768	Δ*ruvABC*::*cam*	B. Michel
N2103	*recC266*::Tn*10*	R. G. Lloyd
CAG12115	*fucP3154*::Tn*10 kan*	CGSC 7428[a]
K797	*phoR79*::Tn*10*	CGSC 6456[a]
STL2694	Δ*xonA300*::*cat*	46
CAG12099	*zef-3129*::Tn*10*	CGSC 7397[a]
N4680	*ruvB71*::*kan*	R. G. Lloyd
STL113	*recJ2052*::Tn*10 kan*	46
Hfr3000	Hayes PO1 *proAB$^+$*	4

[a] Strain supplied from the *E. coli* Genetic Stock Center by M. Berlyn.

moderate deficiencies in recombination and sensitivity to DNA-damaging agents (28, 43). This phenotype of *ruv* mutants is much more pronounced in a *recBC sbcBC* background, suggesting that the RuvABC complex is indispensable for recombination and repair in the RecF pathway (28). This difference in the requirement of two recombinational pathways for RuvABC is, however, poorly understood. The aim of this work was to examine the genetic basis for the extreme recombination deficiency of *recBC sbcBC ruv* mutants.

MATERIALS AND METHODS

Strains. The *E. coli* strains used in this study are listed in Table 1. Most of them are derivatives of AB1157. New strains were constructed by P1 transduction, as described by Miller (36). To obtain *sbcB15 sbcC201* strain LMM965, the parental strain JC7623 was first marked with *recC266*::Tn*10* and then transduced to *recBC$^+$* with *fuc3154*::Tn*10 kan*. The Km^r Tc^s transductants were checked for *recBC$^+$* phenotype by examining the efficiency of plating of the T42*am* phage (which is decreased about 1,000-fold in *recBC$^+$* cells in comparison to *recB* or *recC* mutants) (37). The same procedure was used with the *rus-1* derivative of JC7623 to obtain *sbcB15 sbcC201 rus-1* strain LMM967. To construct a strain that carries only *sbcB15*, the wild-type *sbcC$^+$* allele was cotransduced with *phoR79*::Tn*10* into the *sbcB15 sbcC201* strain LMM965. Strain LMM984 *sbcB15*

rus-1 was constructed in the same way, starting from the *sbcB15 sbcC201 rus-1* strain LMM967. The *sbcC$^+$* phenotype of Tc^r transductants was confirmed by the decreased efficiency of plating of λ phage carrying a 571-bp palindrome (17). λ*pal571* formed plaques on *sbcC$^+$* constructs LMM979 and LMM984 with about 300-fold-lower efficiency than on parental strains carrying mutations in *sbcC*.

Media, growth conditions, and irradiation. Bacteria were grown in Luria-Bertani (LB) medium and on LB plates (36). When required, LB plates were supplemented with the appropriate antibiotics: tetracycline, 10 μg/ml; kanamycin, 25 μg/ml; and chloramphenicol, 15 μg/ml.

In all experiments, bacteria were grown from a single colony in LB medium at 37°C until they reached an optical density at 600 nm of 0.2. For UV experiments, serial dilutions of bacterial cultures were plated on LB plates and irradiated with a dose of UV (254-nm) light of 10 J/m^2. The dose rate was 0.25 J/m^2/s. In experiments with gamma irradiation, bacteria were first exposed to a dose of 200 Gy and then plated for colonies. γ-Irradiation was carried out at 0°C from a ^{60}Co source at a dose rate of 12.5 Gy/s. 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 (36). Inheritance of the chromosomal Pro$^+$ marker was assayed. Donor (Hfr3000) and recipient strains were grown to an optical density at 600 nm of 0.2 at 37°C before being mixed at a ratio of 1:10. Matings were allowed for 30 min. *proAB$^+$* recombinants were selected on M9 plates (36) supplemented with glucose (0.4%), vitamin B$_1$ (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.

TABLE 2. Survival of different *ruv* derivatives after UV irradiation[a]

Strain	Relevant genotype	Avg survival ratio ± SD at 10 J/m^2
AB1157	Wild type	0.89 ± 0.04
LMM10	Δ*ruvABC*	0.051 ± 0.002
JC7623	*recBC sbcBC*	0.73 ± 0.02
LMM864	*recBC sbcBC* Δ*ruvABC*	0.0004 ± 0.00003
JC5519	*recBC*	0.046 ± 0.012
LMM20	*recBC* Δ*ruvABC*	0.035 ± 0.004
LMM965	*sbcBC*	0.69 ± 0.04
LMM966	*sbcBC* Δ*ruvABC*	0.0003 ± 0.0001
LMM979	*sbcB*	0.84 ± 0.05
LMM983	*sbcB* Δ*ruvABC*	0.00039 ± 0.00008
N2364	*sbcC*	0.83 ± 0.05
LMM971	*sbcC* Δ*ruvABC*	0.023 ± 0.003
LMM984	*sbcB rus-1*	0.89 ± 0.06
LMM986	*sbcB rus-1* Δ*ruvABC*	0.68 ± 0.06
LMM997	Δ*xonA*	0.90 ± 0.04
LMM1015	*ruvB*	0.045 ± 0.004
LMM1017	Δ*xonA ruvB*	0.041 ± 0.005
LMM1018	*sbcB ruvB*	0.00062 ± 0.00011
LMM1032	*recJ*	0.54 ± 0.09
LMM1033	*recBC sbcBC recJ*	0.00005 ± 0.00002
LMM1034	*sbcB recJ*	0.54 ± 0.07
LMM1035	*sbcB recJ* Δ*ruvABC*	0.00028 ± 0.00009

[a] In all strains listed, the designation *sbcB* corresponds to the *sbcB15* mutation. Values are averages ± standard deviations of results from three independent experiments. Ratios of the number of colonies on irradiated plates to that on nonirradiated plates are given.

RESULTS AND DISCUSSION

recBC sbcBC mutants of *E. coli* display recombinational repair proficiency similar to that of *rec*$^+$ *sbc*$^+$ cells (18) (compare strains JC7623 and AB1157 in Tables 2 and 3), suggesting that the two recombinational pathways (RecBCD and RecF) are equally efficient in repairing DNA strand breaks. Current models of homologous recombination and recombinational repair imply that the final stage of this process (i.e., the resolution of Holliday intermediates by the RuvABC complex) is common to both pathways (23, 26). However, it was shown that mutations in the *ruv* genes more strongly affect recombination and repair in *recBC sbcBC* mutants than in wild-type cells (28) (Tables 2 and 3).

Since *recBC sbcBC* mutations inactivate three recombination-associated enzymes (RecBCD, ExoI, and SbcCD), we considered the possibility that some of these mutations could be responsible for the extreme repair deficiency that is typical of *recBC sbcBC ruv* strains. In order to test this possibility, we constructed a series of strains in which the *ruvABC* deletion was combined with particular mutations from the *recBC sbcBC* strain JC7623 (Table 1). The recombination proficiency of these strains was tested by measuring their survival after UV irradiation. The result obtained with a *recBC* Δ*ruvABC* mutant was almost identical to those observed with *recBC* and Δ*ruvABC* single mutants; UV survival of all three mutants was decreased about 20-fold in comparison to the wild-type strain (Table 2). This result shows that the combination of these mutations is not responsible for the extreme UV sensitivity of *recBC sbcBC* Δ*ruvABC* cells. In contrast, the *sbcBC* Δ*ruvABC* mutant LMM966 was 2,300-fold and 3,000-fold more sensitive to UV than its parental *sbcBC* strain LMM965 and wild-type strain AB1157, respectively. This high UV sensitivity was al-

most identical to that of the *recBC sbcBC* Δ*ruvABC* strain LMM864.

In further analysis, we wanted to examine the contribution of particular *sbc* mutations to the UV sensitivity of the *sbcBC* Δ*ruvABC* strain. We found that the *sbcB* Δ*ruvABC* mutant LMM983 was as UV sensitive as *sbcBC* Δ*ruvABC* and *recBC sbcBC* Δ*ruvABC* strains (Table 2). On the other hand, the *sbcC* Δ*ruvABC* strain LMM971 displayed moderate UV sensitivity similar to that of the Δ*ruvABC* single mutant. Therefore, we concluded that the *sbcB15* mutation alone is responsible for the extreme repair deficiency observed with *ruv* derivatives of the *recBC sbcBC* strain JC7623. In other words, in the presence of the *sbcB15* mutation, recombinational repair of UV-induced DNA lesions relies on the RuvABC complex much more than in an *sbcB*$^+$ background.

The RuvABC complex has been shown to promote two reactions in vitro, a Holliday junction branch migration reaction catalyzed by RuvAB, and a junction resolution reaction catalyzed by RuvC (49). To determine which of the two activities of RuvABC is critical for the repair proficiency of an *sbcB* mutant, we studied the effect of a *rus-1* mutation on the UV survival of the *sbcB* Δ*ruvABC* strain. The *rus-1* mutation was previously shown to induce synthesis of the RusA protein, which can substitute for RuvABC in the process of Holliday junction resolution but does not catalyze branch migration (8, 32, 42). Our results showed that both *sbcB rus-1* strain LMM984 and *sbcB rus-1* Δ*ruvABC* strain LMM986 were as UV resistant as the wild-type strain (Table 2). We therefore inferred that Holliday junction resolution is indispensable for successful recombinational repair in the *sbcB* mutant of *E. coli*.

The results described above suggest that interfering with ExoI function makes DNA repair more dependent on the RuvABC complex. The *sbcB15* mutation is known to abolish the activity of ExoI but does not prevent synthesis of the mutant protein (38). Although it is generally assumed that the

TABLE 3. Survival of different Δ*ruvABC* derivatives after γ-irradiation[a]

Strain	Relevant genotype	Avg survival ratio ± SD at 200 Gy
AB1157	Wild type	0.64 ± 0.07
LMM10	Δ*ruvABC*	0.067 ± 0.020
JC5519	*recBC*	0.0007 ± 0.0001
LMM20	*recBC* Δ*ruvABC*	0.0004 ± 0.00005
JC7623	*recBC sbcBC*	0.70 ± 0.18
LMM864	*recBC sbcBC* Δ*ruvABC*	0.00002 ± 0.00001
LMM965	*sbcBC*	0.48 ± 0.12
LMM966	*sbcBC* Δ*ruvABC*	0.0003 ± 0.0001
LMM979	*sbcB*	0.51 ± 0.05
LMM983	*sbcB* Δ*ruvABC*	0.0011 ± 0.0006
N2364	*sbcC*	0.48 ± 0.02
LMM971	*sbcC* Δ*ruvABC*	0.033 ± 0.006
TNM734	*recBC sbcBC rus-1*	0.60 ± 0.05
LMM867	*recBC sbcBC rus-1* Δ*ruvABC*	0.37 ± 0.02
LMM967	*sbcBC rus-1*	0.44 ± 0.05
LMM969	*sbcBC rus-1* Δ*ruvABC*	0.37 ± 0.02
LMM984	*sbcB rus-1*	0.73 ± 0.09
LMM986	*sbcB rus-1* Δ*ruvABC*	0.49 ± 0.09

[a] In all strains listed, the designation *sbcB* corresponds to the *sbcB15* mutation. Values are averages ± standard deviations of results from at least three independent experiments. Ratios of the number of colonies formed by irradiated cells to that formed by nonirradiated cells are given.

effect of the *sbcB15* mutation on DNA recombination is due to inactivation of ExoI, some results suggest that SbcB15 protein additionally modulates the recombinational process by binding to 3′ DNA ends (6, 39). This binding could protect 3′ ends from the action of other nucleases that have affinity for the same DNA substrate.

To get further insight into the functional relationship between ExoI and RuvABC in UV repair, we tested the effect of the Δ*xonA300* mutation on DNA repair in a strain deficient for Ruv activity. The Δ*xonA300* mutation eliminates the whole *sbcB* coding sequence, leading to complete absence of ExoI protein (39). As shown in Table 2, the Δ*xonA ruvB* mutant LMM1017 was indistinguishable from the *xonA⁺ ruvB* strain LMM1015; both strains displayed moderate UV sensitivity typical of single *ruv* mutants. However, when Δ*xonA* was replaced with the *sbcB15* mutation from JC7623 (see strains LMM1001 and LMM1018 in Table 1), the presence of the *ruvB* mutation dramatically increased UV sensitivity (Table 2). These results indicate that elimination of ExoI activity by itself is not responsible for the extreme DNA repair defect observed with *sbcB15 ruv* mutants. It is possible that the presence of the mutant SbcB protein modifies recombinational repair in such a way that it requires Holliday junction resolution as an obligatory step.

The pronounced recombinational deficiency of *recBC sbcBC ruv* mutants in comparison to *rec⁺ sbc⁺ ruv* strains suggests that an unknown intrinsic property of the RecF pathway makes recombination more dependent on Ruv functions. However, our results with *sbcBC ruv* and *sbcB ruv* strains indicate that a similar dependence on Ruv exists even in the presence of functional RecBCD enzyme. One possible explanation for these results is that the *sbcB* mutation redirects recombination from the RecBCD to the RecF pathway, thereby making the latter pathway dominant. To test this possibility, we compared the effect of a *recJ* mutation on UV survival in wild-type, *recBC sbcBC*, *sbcB*, and *sbcB* Δ*ruvABC* backgrounds.

The RecJ protein is known to be required for the efficient initiation of recombination in the *recBC sbcBC* background (i.e., on the RecF pathway) (11). Consistent with the previous results of other authors (31), a *recJ* mutation caused strongly pronounced UV sensitivity in a *recBC sbcBC* strain while having only a slight effect in the wild-type background (compare strains LMM1032 and LMM1033 in Table 2). The repair proficiency of the *sbcB recJ* mutant was the same as that of the *recJ* single mutant and about 10⁴-fold higher than that observed with the *recBC sbcBC recJ* mutant. These results indicate that in *recBC⁺ sbcB15* strains, the RecBCD pathway remains fully active in recombinational repair. The *sbcB recJ* Δ*ruvABC* strain LMM1035 displayed extreme UV sensitivity quite similar to that of the *recBC sbcBC* Δ*ruvABC*, *sbcBC* Δ*ruvABC*, and *sbcB* Δ*ruvABC* mutants (Table 2). A similar set of results was obtained with strains containing a *recF* mutation instead of *recJ* (data not shown). It seems, therefore, that the *ruv*-associated UV repair defect observed in *recBC sbcBC*, *sbcBC*, and *sbcB* backgrounds depends directly on the *sbcB* mutation, regardless of the recombinational pathway that is active in the cell.

It was recently shown that the RuvABC complex has a dual repair function: in addition to its well-known role in the postsynaptic stage of recombinational repair, it also participates in the removal of stalled replication forks. It was proposed that replication forks arrested at different obstacles in

DNA (e.g., DNA-bound proteins and DNA secondary structures) could be reversed and transformed into Holliday structures and then cleaved by the action of the RuvABC proteins (41). The double-stranded DNA ends thus created could be used by RecBCD enzyme to initiate homologous recombination, leading to replication fork restoration. A similar scenario was shown to occur in UV-irradiated cells, in which replication forks are frequently arrested at unrepaired pyrimidine dimers (34). Moreover, it was proposed that the majority of double-strand breaks (DSBs) in UV-irradiated cells arise from RuvABC action on reversed replication forks.

In light of these results, the strong requirement for RuvABC resolvase observed in UV-irradiated *sbcB15* mutants could reflect the need for Holliday junction resolution in either the presynaptic or postsynaptic stage of recombinational repair. To discriminate between these two possibilities, we first examined the survival of different Δ*ruvABC* and *sbcB15* derivatives after exposure to gamma irradiation. It is known that γ-irradiation produces various types of DNA damage, including single- and double-strand DNA breaks, oxidized purine and pyrimidine bases, abasic sites, and DNA-protein crosslinks (reviewed in references 47 and 48). Among the lesions listed, DSBs are considered the primary cause of cellular lethality after ionizing radiation (16, 19). DSBs can arise directly from the radiation energy deposited in the DNA molecule or indirectly following attack on the DNA of free radicals produced from water. In addition, a significant number of DSBs appear during attempted excision repair at sites of clustered DNA lesions (7). Also, they can result from DNA polymerase running into unrepaired single-strand breaks (25). In all cases mentioned, DSBs are subject to recombinational repair, during which the RuvABC complex is expected to act only at the postsynaptic stage.

The results of experiments with γ-irradiation are presented in Table 3. The dose of 200 Gy moderately reduced the survival of Δ*ruvABC* strain LMM10 while having a much stronger effect on the *recBC* mutant JC5519. The difference in survival between the two strains was almost 100-fold. These results show that in the wild-type background, the RecBCD enzyme has a more important role in γ-irradiation repair than the RuvABC complex. The *recBC* Δ*ruvABC* double mutant LMM20 was as sensitive as the *recBC* single mutant. The lack of additivity of the *recBC* and Δ*ruvABC* mutations indicates that the RuvABC proteins act in the RecBCD pathway during repair of γ-irradiation-induced DSBs. Also, these results show that in wild-type cells, the majority of DSB repair after ionizing radiation does not depend on RuvABC.

As expected, the Δ*ruvABC* mutation had a much more profound effect in the *recBC sbcBC* than in the wild-type background; the *recBC sbcBC* Δ*ruvABC* mutant was about 3,000-fold more sensitive to γ-irradiation than its *rec⁺ sbc⁺* Δ*ruvABC* counterpart (Table 3). Also, the *sbcBC* Δ*ruvABC* and *sbcB* Δ*ruvABC* mutants showed significantly higher γ-irradiation sensitivity (220-fold and 60-fold, respectively) than the single Δ*ruvABC* mutant. The pronounced repair defect of the three *sbcB ruv* derivatives was rectified by the *rus-1* mutation. Taken together, these results indicate that the *sbcB15* mutation generally increases the requirement for RuvABC resolvase in γ-irradiation repair. However, the 15-fold and 55-fold-higher γ-irradiation resistance of the *sbcBC* Δ*ruvABC* and *sbcB* Δ*ruvABC*

TABLE 4. Conjugational recombination with different
Δ*ruvABC* recipient strains

Recipient strain	Relevant genotype[a]	Relative viability[b]	Avg relative yield of recombinants[c] ± SD
AB1157	Wild type	1	1
LMM10	Δ*ruvABC*	0.62 ± 0.02	0.3 ± 0.12
JC5519	*recBC*	0.29 ± 0.03	0.0032 ± 0.0013
LMM20	*recBC* Δ*ruvABC*	0.25 ± 0.04	0.0026 ± 0.0007
JC7623	*recBC sbcBC*	0.59 ± 0.07	1
LMM864	*recBC sbcBC* Δ*ruvABC*	0.14 ± 0.01	0.022 ± 0.007
LMM965	*sbcBC*	0.72 ± 0.08	1
LMM966	*sbcBC* Δ*ruvABC*	0.21 ± 0.03	0.063 ± 0.004
LMM979	*sbcB*	0.92 ± 0.09	0.77 ± 0.18
LMM983	*sbcB* Δ*ruvABC*	0.25 ± 0.03	0.13 ± 0.02
N2364	*sbcC*	1	1
LMM971	*sbcC* Δ*ruvABC*	0.68 ± 0.11	0.24 ± 0.05

[a] In all strains listed, the designation *sbcB* corresponds to the *sbcB15* mutation.
[b] Viability is given relative to the number of CFU per milliliter in the cultures of the control recipient strain AB1157, which averaged 5.7×10^7.
[c] Yields of recombinants are relative to the control strain AB1157 and have been corrected for any deficiency in the viability of the recipient cells. The average yield for the control strain AB1157 was 6.4×10^5 per ml of the mating mixture. Values are averages ± standard deviations of results from three independent experiments.

strains, respectively, in comparison to the *recBC sbcBC* Δ*ruvABC* mutant indicates that the RecBCD function and, to a lesser extent, also the SbcC function in the former strains decrease the necessity for RuvABC in γ-irradiation repair. This finding suggests that, even in the presence of the *sbcB* mutation, the postsynaptic stage of DSB repair is (at least partly) independent of RuvABC proteins.

To further study the role of the RuvABC complex in the postsynaptic stage of recombination in the *sbcB15* background, we investigated conjugational recombination in different Δ*ruvABC* and/or *sbcB15* derivatives. During conjugational crosses, a single strand of Hfr DNA is transferred to the F⁻ recipient, where it provides a template for DNA synthesis (15). When mating terminates, the transferred DNA is released as a linear fragment that is subjected to recombinational exchanges with the circular recipient chromosome. The vast majority of recombinants in such crosses arise from recombinational events initiated at the ends of donor DNA by the RecBCD enzyme (10, 44). The RuvABC complex acts late in this process by cleaving the Holliday junctions formed after RecA-mediated DNA strand exchange.

As shown in Table 4, the Δ*ruvABC* mutation mildly decreased the frequency of conjugational recombination. In contrast, recombination was drastically reduced in the *recBC* recipient. Introducing the Δ*ruvABC* mutation into the *recBC* background had no additional effect on recombination frequency, indicating that the RecBCD and RuvABC proteins act in the same recombinational pathway. The recombination frequency of the *recBC sbcBC* Δ*ruvABC* mutant was approximately 10-fold lower than that of the single Δ*ruvABC* mutant, quite a modest difference in comparison with those obtained with the same strains after UV and γ-irradiation (compare results in Tables 2, 3, and 4).

Also in contrast to the radiation experiments, the recombination proficiency of the *recBC sbcBC* Δ*ruvABC* mutant was almost 10-fold higher than that of the *recBC* or *recBC* Δ*ruv-ABC* strain. These differences between conjugational experiments on the one hand and radiation experiments on the other most probably reflect the differences between the DNA substrates present in the experimental systems used. As in the γ-irradiation experiment, the *sbcBC* Δ*ruvABC* and *sbcB* Δ*ruv-ABC* mutants displayed recombination frequencies somewhat higher than that of the *recBC sbcBC* Δ*ruvABC* mutant. Furthermore, the *sbcB* Δ*ruvABC* cells had slightly lower recombination than the Δ*ruvABC* single mutant. Taking into account all results described, it appears that the *sbcB15* mutation alone does not significantly change the role of the RuvABC complex in conjugational crosses and, by inference, does not affect its role in the postsynaptic stage of recombination in general. However, we cannot exclude the possibility that the *sbcB15* mutation to a certain extent alters the postsynaptic stage of recombination in a *recBC sbcC* background.

To summarize, our results obtained in γ-irradiation and conjugational experiments suggest that the postsynaptic stage of recombination in the *sbcB15* single mutant is largely independent of RuvABC proteins. Therefore, the extreme DNA repair defect that was observed with the *sbcB15* Δ*ruvABC* mutant after UV irradiation must be attributed to a block in an earlier phase of recombination. These results are in accord with the model which proposes that in UV-irradiated cells, the RuvABC complex acts on Holliday junctions made from reversed replication forks. Perhaps the SbcB15 protein alters the processing of such Holliday junctions and makes their removal more dependent on the action of the RuvABC resolvase. The same model could explain the increased requirement for Ruv-ABC in γ-irradiated *sbcB15* cells.

Although γ-radiation is often used as a test system to study recombinational repair of DSBs, the repair of other γ-irradiation-induced lesions could also require the action of recombination proteins. It is possible that some types of base damage that appear after ionizing radiation have the same effect on DNA replication as that proposed for UV-induced pyrimidine dimers, the reversal of replication forks. If so, in γ-irradiated *E. coli* cells, the RuvABC complex would be expected to act both during the repair of DSBs and on removal of regressed replication forks. This could explain our finding that the *recBC sbcBC* Δ*ruvABC* mutant is more sensitive to γ-irradiation than the *recBC* Δ*ruvABC* mutant (Table 3); in addition to a block in DSB repair, the first strain could also suffer from defective processing of regressed replication forks.

Concluding remarks. The recent model of recombinational repair in UV-irradiated cells (34) proposes several pathways for the processing of Holliday junctions formed by replication fork regression. In addition to resolution by the RuvABC complex (Fig. 1c), a Holliday junction could be transformed back to a replication fork by simple forward branch migration (catalyzed by RuvAB or RecG helicase) or by exonucleolytic degradation of the double-stranded DNA arm extruded upon replication fork regression (Fig. 1b). Previous studies by McGlynn and Lloyd (34) suggest that the RecBCD enzyme has no access to the double-stranded DNA tail made by fork reversal. Thus, exonucleolytic degradation of this tail must rely on other nucleases. However, RecBCD was proposed to have a key role in DNA repair following RuvABC-mediated fork cleavage (34) (Fig. 1c and d).

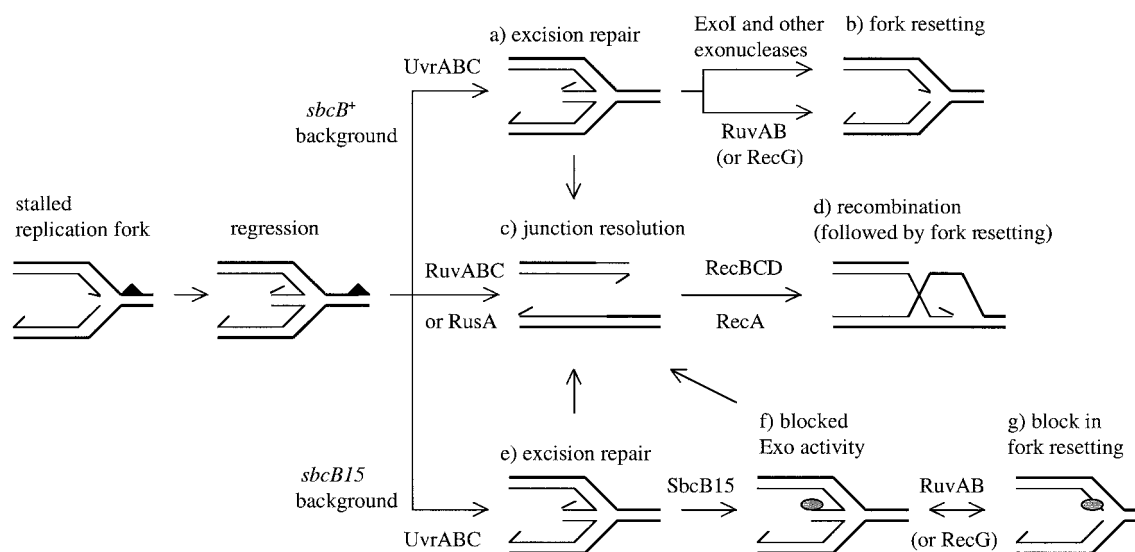Our results suggest that in the presence of the *sbcB15* mu-

FIG. 1. Effect of the SbcB15 protein on the repair of replication forks stalled at pyrimidine dimers. The upper part of the figure represents molecular events occurring in UV-irradiated wild-type (*sbcB*⁺) cells. The lower part pictures reactions occurring in the *sbcB15* background. Reactions shown in the middle part of the figure are common to both backgrounds. The solid triangle and the shaded oval represent a pyrimidine dimer and the SbcB15 protein, respectively. The directions of DNA synthesis within replication forks are indicated by arrows. Details of the model are discussed in the text.

tation, RuvABC-mediated cleavage of Holliday junctions is the principal pathway for the repair of blocked replication forks. The simplest explanation for this finding would be that inactivation of *sbcB* function prevents exonucleolytic cleavage of the duplex DNA end formed upon replication fork reversal. However, the absence of any additional effect of the Δ*xonA* mutation in the *ruv* background suggests that ExoI does not play a crucial role in replication fork resetting. It is more likely that the *sbcB15* mutation modulates the repair of blocked replication forks in an indirect way, possibly through the binding of the mutant SbcB15 protein to the duplex DNA end, which could prevent the action of other nucleases (Fig. 1f).

Such an explanation implies that the extruded duplex arm of a reversed replication fork must expose a single-stranded 3′ end as the substrate for the SbcB protein. The free 3′ end could arise from DNA unwinding catalyzed by RecQ or some other recombination-associated helicase. The binding of SbcB15 to the 3′ end of a duplex arm might affect not only the exonucleolytic processing of the regressed fork, but perhaps also the restoration of the replication fork by forward branch migration (Fig. 1g). In the latter case, SbcB15 may prevent completion of forward branch migration and/or interfere with the restart of DNA replication from the restored fork-like structure. In such a situation, the RuvABC-mediated cleavage of the regressed fork followed by recombinational repair would be the only way to circumvent the block in DNA synthesis and to obtain viable chromosomes.

The cleavage of a regressed replication fork produces a DNA duplex which can be used by either the RecBCD or the RecF pathway to initiate recombinational repair and to restore a replication fork (6). The relatively high UV resistance obtained with *sbcB15 recJ* (Table 2) and *sbcB15 recF* (not shown) mutants suggests that the SbcB15 protein does not interfere with RecBCD-dependent recombination. This implies that

SbcB15 does not prevent the binding of RecBCD to duplex DNA. Perhaps RecBCD is capable of removing SbcB15 from the 3′ end either immediately upon binding to DNA or later, during its temporary exonucleolytic activity on the 3′ strand.

As already mentioned, upon encountering a Chi sequence, RecBCD enzyme changes its mode of action from a powerful DNA degradase to a recombinase that produces a recombinogenic 3′ ssDNA tail. At the same time, RecBCD loads the RecA protein onto the exposed ssDNA, which enables the subsequent synaptic stage of recombination (2, 3). It was also shown that the RecBCD-mediated loading of the RecA protein protects the 3′ ssDNA tail from digestion by ExoI (2). Therefore, it is very likely that the activity of the RecBCD enzyme prevents repeated binding of the SbcB15 protein in a similar way once it has been removed from the 3′ DNA end. In such conditions, the reactions initiated by RecBCD can proceed normally to form a D-loop that serves as a substrate for PriA-mediated replication restart (40) (Fig. 1c and d).

Our results obtained in conjugal crosses indicate that the postsynaptic stage of recombination is more dependent on the RuvABC proteins in the *recBC sbcBC* mutant than in the wild-type cells. The higher necessity for RuvABC in the *recBC sbcBC* background can be attributed not solely to *sbcB15* but rather to a joint effect of all the mutations present. We speculate that in the absence of a functional RecBCD enzyme, the mutant SbcB15 protein binds to a recombinogenic 3′ DNA end, thereby making the final stage of recombination more dependent on the RuvABC resolvase. Such binding could occur if we assume that RecFOR-catalyzed loading of the RecA protein is not as efficient as that catalyzed by RecBCD. Perhaps SbcB15 bound to a 3′ DNA end interferes with DNA synthesis within the D-loop, which makes the recombination intermediate unstable and calls for Holliday junction resolution. However, the present results do not offer clear evidence

for this assumption, and further work will be needed to address this issue.

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