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recD sbcB sbcD Mutants Are Deficient in Recombinational Repair of UV Lesions by RecBC

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In *recD sbcB sbcD* mutants, repair of UV-irradiated DNA is strongly RecF dependent, indicating that RecBC is inactive. This finding suggests that exonuclease V, exonuclease I (SbcB), and the SbcCD nuclease play a redundant role in vivo, which is essential for the recombination activity of the RecBC complex during UV repair.

Homologous recombination can be initiated in *Escherichia coli* by either the RecBCD complex or the RecF, RecO, and RecR proteins (reviewed in reference 8). The two pathways of recombination require different substrates, which are DNA double-stranded ends for RecBCD and gapped DNA for RecF. Since homologous recombination plays an important role in the repair of UV lesions, recombination mutants are sensitive to UV irradiation. Inactivating either *recBC* or *recF* leads to a decrease in the survival of UV-irradiated cells, indicating that both types of substrates are formed upon UV irradiation (13). In the absence of RecBC, *sbcB* and *sbcCD* suppressor mutations allow the RecF, RecO, and RecR proteins to catalyze recombination initiated at double-stranded ends (reviewed in reference 3). In contrast with *recBC* mutants, *recD* mutants lack only the exonuclease V activity of RecBCD and they are proficient for homologous recombination and resistant to UV irradiation. These findings indicate that the RecBC subunits are sufficient for recombination (2). Similarly, a *recB*(Ts) *recC*(Ts) strain, deficient for exonuclease V activity at low temperature, can be transduced (6, 12). Since a *recB*(Ts) *recC*(Ts) *recF* strain can also be transduced (1), recombination in the *recB*(Ts) *recC*(Ts) strain at low temperature is likely to be catalyzed by the remaining activity of RecBC (7) and not by the RecF pathway. However, we observed that in *recB*(Ts) *recC*(Ts) *sbcB sbcC* strains, introduction of a *recF* mutation abolished P1 transduction, showing that in this strain P1 transduction is catalyzed exclusively by the RecF pathway (our unpublished data). This finding suggests that the *sbcB sbcCD* mutations affect the residual recombination activity of RecBC in *recB*(Ts) *recC*(Ts) strains. To test whether this could result from the exonuclease V defect of *recB*(Ts) *recC*(Ts) mutants at low temperature, we used *recD* derivatives. Recombination proficiency was tested by measuring survival after UV irradiation. See Table 1 for the strains used.

Serial dilutions of exponential cultures (optical densities ≈ 0.5) of different strains were plated. Plates were UV irradiated at 40 J/m^2 and incubated for 24 h at 37°C. The ratios of the numbers of colonies on irradiated plates to those on nonirradiated plates were calculated. In *recF*-proficient strains, inactivation of the *sbcB*, *sbcD*, and *recD* genes did not affect significantly UV survival (Table 2). In *recF* mutants, UV recombinational repair was mediated by RecBCD (compare JJC979 and JJC980 in Table 2). The effects of various mutations on UV survival in *recF* strains therefore reflect a role for the corresponding genes in RecBCD-mediated recombinational repair. Inactivation of *recD* did not affect UV repair significantly (9) (compare JJC979 with JJC999 in Table 2), indicating that RecBC is proficient for recombinational repair in *recF* strains. Inactivation of *sbcB* or *sbcD* in the *recF recD* mutant only slightly decreased UV resistance (compare JJC1000, JJC1001, and JJC999 in Table 2). However, the simultaneous inactivation of *sbcB* and *sbcD* was much more dramatic, as UV repair was strongly decreased (≈100-fold) in the *recF recD sbcB sbcD* strain (JJC1003) (Table 2). The observation that UV recombinational repair is RecF dependent in a *recD sbcB sbcD* strain indicates that RecBC-mediated repair is inefficient in this strain. Therefore, the presence of either SbcB or SbcCD is essential for the UV repair catalyzed by RecBC in the absence of exonuclease V. Participation of SbcB or SbcCD in the repair of UV lesions by the RecBCD complex could also be observed in exonuclease V-proficient

TABLE 1. Strains

Strain	Relevant genotype	Origin or reference
FG252	*sbcD300*::*kan*	R. Lloyd
JJC40	Wild type (AB1157 *hsdR*)	Laboratory Stock
JJC273	*recD1901*::Tn*10*	12
JJC685	*recF332*::Tn*3*	1
JJC777	*recB*::Tn*10* (pDWS2)	1
JJC885	*recD1901*::Tn*10 sbcD300*::*kan*	P1 FG252 × JJC273
JJC889	Δ*sbcB*	1
JJC890	*recD1901*::Tn*10* Δ*sbcB*	P1 JJC889 × JJC273
JJC979	*recF332*::Tn*3*	P1 JJC685 × JJC40
JJC980	*recF332*::Tn*3 recB268*::Tn*10*	P1 JJC777 × JJC979
JJC999	*recD1901*::Tn*10 recF332*::Tn*3*	P1 JJC685 × JJC273
JJC1000	*recD1901*::Tn*10 sbcD300*::*kan* *recF332*::Tn*3*	P1 JJC685 × JJC885
JJC1001	*recD1901*::Tn*10* Δ*sbcB* *recF332*::Tn*3*	P1 JJC685 × JJC890
JJC1002	*recD1901*::Tn*10* Δ*sbcB* *sbcD300*::*kan*	P1 JJC889 × JJC885
JJC1003	*recD1901*::Tn*10* Δ*sbcB* *sbcD300*::*kan recF332*::Tn*3*	P1 JJC685 × JJC1002
JJC1004	Δ*sbcB sbcD300*::*kan*	P1 FG252 × JJC889
JJC1007	Δ*sbcB sbcD300*::*kan recF332*::Tn*3*	P1 JJC685 × JJC1004

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TABLE 2. Survival of a *recD sbcB sbcD* strain after UV irradiation depends on RecF

Strain	Relevant genotype	Survival at 40 J/m^2[a]
JJC40	Wild type	0.89 ± 0.32
JJC273	*recD*	0.46 ± 0.11
JJC1004	*sbcB sbcD*	0.53 ± 0.17
JJC1002	*recD sbcD sbcD*	0.43 ± 0.22
JJC979	*recF*	0.038 ± 0.011
JJC980	*recF recB*	0.000005 ± 0.000002
JJC999	*recF recD*	0.028 ± 0.025
JJC1000	*recF recD sbcD*	0.013 ± 0.001
JJC1001	*recF recD sbcB*	0.013 ± 0.004
JJC1003	*recF recD sbcB sbcD*	0.000095 ± 0.000041
JJC1007	*recF sbcB sbcD*	0.0047 ± 0.0023

[a] Values are averages ± standard deviations of results from three to five independent experiments. Ratios of the numbers of colonies on irradiated plates to those on nonirradiated plates are given.

strains: in the absence of both SbcB and SbcCD, RecBCD-mediated UV repair decreased 10-fold (compare JJC979 and JJC1007 in Table 2). This result suggests a redundant function for SbcB and SbcCD in RecBCD-mediated recombination.

The combination of *sbcB sbcCD* mutations was previously reported to decrease the exonuclease V action of RecBCD (11). SbcB and SbcCD were proposed to be essential for the blunting of DNA ends prior to RecBCD binding. We show here that SbcB or SbcCD is essential for RecBC-mediated repair when exonuclease V is inactive, since in *recD sbcB sbcD* strains, UV recombinational repair is entirely RecF dependent. This redundant function of exonuclease V, exonuclease I, and SbcCD nuclease in homologous recombination may also be responsible for the defect in RecBC-mediated recombination in a *recB*(Ts) *recC*(Ts) *sbcB sbcC* strain (our unpublished data). SbcB and SbcCD are not the only nucleases that play a role in RecBC-catalyzed recombination in the absence of RecD. Inactivation of the RecJ nuclease strongly increases the UV sensitivity of *recD* mutants (9, 10) and causes the lethality of *rep recD* strains (12), which suggests that RecJ is essential both for RecBC and RecFOR UV repair and for RecBC-mediated recombination in *rep* mutants. SbcB and SbcCD differ from RecJ in that their absence allows RecF-mediated recombination. RecJ and SbcB are single-stranded exonucleases that degrade DNA in the 5′-to-3′ and 3′-to-5′ directions, respectively. SbcCD is a double-stranded DNA exonuclease with an endonucleolytic activity directed to palindromic DNA (4). Our results, combined with the properties of *recJ* mutants, indicate that (i) SbcB and SbcCD proteins have redundant actions on UV-generated DNA ends, probably to process 3′ protruding ends; (ii) the simultaneous processing of 3′ and 5′ DNA ends is essential for the repair by RecBC, as both RecJ and SbcB or SbcCD are required; and (iii) RecBCD can act on both types of DNA ends, as the presence of the RecD subunit relieves the requirement for RecJ and for SbcB or SbcCD. In vitro, RecBC appears to have a lower affinity for duplex ends than RecBCD and to bind better to some overhangs than others (5). Similarly, different types of DNA ends may be required for the binding of RecBC and RecBCD in vivo, leading to a specific requirement for enzymes that process double-stranded DNA ends.

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