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DNA Helicases in Recombination and Repair: Construction of a Δ*uvrD* Δ*helD* Δ*recQ* Mutant Deficient in Recombination and Repair

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DNA helicases play pivotal roles in homologous recombination and recombinational DNA repair. They are involved in both the generation of recombinogenic single-stranded DNA ends and branch migration of synapsed Holliday junctions. *Escherichia coli* helicases II (*uvrD*), IV (*helD*), and RecQ (*recQ*) have all been implicated in the presynaptic stage of recombination in the RecF pathway. To probe for functional redundancy among these helicases, mutant strains containing single, double, and triple deletions in the *helD*, *uvrD*, and *recQ* genes were constructed and examined for conjugational recombination efficiency and DNA repair proficiency. We were unable to construct a strain harboring a Δ*recQ* Δ*uvrD* double deletion in a *recBC sbcB*(*C*) background (RecF pathway), suggesting that a Δ*recQ* deletion mutation was lethal to the cell in a *recBC sbcB*(*C*) Δ*uvrD* background. However, we were able to construct a triple Δ*recQ* Δ*uvrD* Δ*helD* mutant in the *recBC sbcB*(*C*) background. This may be due to the increased mutator frequency in Δ*uvrD* mutants which may have resulted in the fortuitous accumulation of a suppressor mutation(s). The triple helicase mutant *recBC sbcB*(*C*) Δ*uvrD* Δ*recQ* Δ*helD* was severely deficient in Hfr-mediated conjugational recombination and in the repair of methylmethane sulfonate-induced DNA damage. This suggests that the presence of at least one helicase—helicase II, RecQ helicase, or helicase IV—is essential for homologous recombination and recombinational DNA repair in a *recBC sbcB*(*C*) background. The triple helicase mutant was recombination and repair proficient in a *rec*$^+$ background. Genetic analysis of the various double mutants unmasked additional functional redundancies with regard to conjugational recombination and DNA repair, suggesting that mechanisms of recombination depend both on the DNA substrates and on the genotype of the cell.

Study of a variety of different recombination processes has led to the identification of several loci in *Escherichia coli* capable of affecting efficiencies of homologous recombination (6). One of the processes utilized extensively to study the genetics of recombination in *E. coli* has been Hfr-mediated recombination (42). In a wild-type cell strain conjugational recombination depends not only on the product of the *recA* gene but also on the active gene products of *recBC* (6, 42). However, the decreased recombination frequency in a *recBC* mutant can be suppressed by the extragenic suppressor mutations *sbcBC* and *sbcA* (3, 45). The *sbcB* mutation inactivates exonuclease I (21). The *sbcC* mutation is required, in addition to the *sbcB* mutation, for suppression of the recombination-deficient phenotype of *recBC* cells (24). The *sbcBC* mutations activate an alternate recombination pathway traditionally referred to as the RecF pathway of recombination (6). The *sbcA* suppression is attributed to the activation of exonuclease VIII and RecT protein encoded by the *recE* gene on the rac prophage (11, 22). The activated pathway is referred to as the RecE pathway of recombination (6, 45). Historically, genes encoding proteins involved in recombination have been grouped together in one of these pathways (6, 12, 42). However, genetic studies have demonstrated that the mechanisms used to generate recombinant DNA molecules are dependent on both the initiating DNA substrate and the genetic background of the cell (6, 17, 30).

At the molecular level, recombination reactions can be conveniently subdivided into three stages: (i) the formation of a single-stranded DNA substrate on which RecA protein polymerizes to form a nucleoprotein complex, (ii) catalysis of homologous pairing by the RecA-DNA filament, and (iii) the resolution of DNA strands involved in homologous recombination leading to the formation of recombinant molecules (6, 28). The biochemical activities essential for these various steps are likely to include nucleases, DNA helicases, DNA-binding proteins, proteins mediating strand exchange, proteins mediating resolution of the heteroduplex molecules, and enzymes involved in general DNA metabolism (6, 31).

Recently, much progress has been made in our understanding of the mechanisms of recombination and the proteins that catalyze this process (18, 36). For *E. coli* many of the enzymes required for recombination have been isolated, and their detailed properties and functions are being determined (18, 36). DNA helicase involvement in recombination has been suggested for both the initiation and the resolution of recombination events (6). A common theme for presynaptic helicase involvement in recombination is that a combination of helicase and nuclease activities may provide the single-stranded DNA substrate required by the RecA protein to initiate homologous pairing (28, 46). Such a coupling of activities has been suggested for the RecQ helicase, helicase IV, helicase II, and the RecJ nuclease (19, 28, 33, 46). Functionally, the three helicases are very similar (32), and the involvement of more than one helicase in a recombination pathway is plausible. Helicase II, RecQ helicase, and helicase IV are all 3′-to-5′ helicases that are involved in the RecF pathway of recombination (28, 33). Hence, functional overlap, masking potential interactions, could be one of the reasons that the single helicase mutants do not exhibit an extreme deficiency in recombination or in DNA

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TABLE 1. Bacterial strains and plasmids

Strain[a] or plasmid	Significant features	Source, reference, or derivation
Strains		
C600	*supE44 hsdR thi-1 thr-1 leuB6 lacY1 tonA21* r_{k+} m_{k+}	1
HB101	*supE44 hsd20* r_{B^-} m_{B^-} *recA13 ara14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*	5
JC158	*lacI22* λ^- *HfrP01 serA6 spoT1 thi-1*	7
AB1157	F^- *thr-1 leuB6 thi-1 lacY1 galK2 ara14 xyl5 mtl-1 proA2 his4 argE3 rpsL3* (Sm^r) *tsx-33 supE44 kdgK51*	13
AB1157 derivatives		
SWM1001	Δ*helD*::*cam*	33
SWM1002	Δ*uvrD*::*tet*	33
SWM1003	Δ*recQ*::*kan*	P1.SWM2003 × AB1157 to Kan^r
SWM1010	Δ*helD*::*cam* Δ*uvrD*::*tet*	33
SWM1011	Δ*helD*::*cam* Δ*recQ*::*kan*	P1.SWM2003 × SWM1001 to Kan^r
SWM1050	Δ*uvrD*::*tet* Δ*recQ*::*kan*	P1.SWM2003 × SWM1002 to Kan^r
SWM1101	Δ*helD*::*cam* Δ*uvrD*::*tet* Δ*recQ*::*kan*	P1.SWM2003 × SWM1010 to Kan^r
JC7623	*recB21 recC22 sbcB15*	9
JC7623 derivatives		
SWM2001	Δ*helD*::*cam*	33
SWM2002	Δ*uvrD*::*tet*	33
SWM2003	Δ*recQ*::*kan*	This study
SWM2010	Δ*helD*::*cam* Δ*uvrD*::*tet*	33
SWM2011	Δ*helD*::*cam* Δ*recQ*::*kan*	P1.SWM2003 × SWM2001 to Kan^r
SWM2050(a)[b]	Δ*uvrD*::*tet* Δ*recQ*::*kan* *recQ*$^+$	P1.SWM2003 × SWM2002 to Kan^r
SWM2101	Δ*helD*::*cam* Δ*uvrD*::*tet* Δ*recQ*::*kan*	P1.SWM2003 × SWM2010 to Kan^r
SWM2101(a)[b]	Δ*helD*::*cam* Δ*uvrD*::*tet* Δ*recQ*::*kan* *recQ*$^+$	P1.SWM2003 × SWM2010 to Kan^r
Plasmids		
pKD151	*bla*$^+$ *recQ*$^+$	38
pKD151Δ*recQ*::*kan*	*bla*$^+$ Δ*recQ*::*kan*	This study

[a] All AB1157 derivatives are isogenic with AB1157 except for the indicated markers. SWM1001, SWM1002, SWM1010, SWM2001, SWM2002, and SWM2010 were previously designated AB1157ΔH4, AB1157ΔH2, AB1157ΔH2ΔH4, JC7623ΔH4, JC7623ΔH2, and JC7623ΔH2ΔH4, respectively.
[b] Has two copies of the *recQ* gene, i.e., a wild-type copy and a deletion-insertion copy.

repair reactions associated with recombination (25). Earlier studies have shown that a functional overlap does exist between helicases II and IV in a *recBC sbcBC* strain background (33). The Δ*helD* Δ*uvrD* deletion mutant decreases recombination 100-fold in a RecF background. However, the Δ*helD* Δ*uvrD* mutant shows no effect on DNA repair in the RecF background, as monitored by sensitivity to methylmethane sulfonate (MMS) (33).

Because of their functional similarities, and the lack of an extreme phenotype as single mutants, it was of interest to investigate the functional interactions between RecQ helicase, helicase IV, and helicase II. In this article we report the construction of single, double, and triple helicase (*helD*, *uvrD*, and *recQ*) mutants in different backgrounds and provide evidence that a Δ*recQ* Δ*uvrD* deletion mutation is synthetically lethal in a *recBC sbcBC* background. We also show that a triple helicase mutation (Δ*helD* Δ*uvrD* Δ*recQ*) has no effect on recombination frequency in a wild-type background but decreases recombination and repair proficiency substantially in the *recBC sbcBC* background.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are listed in Table 1. All of the strains involved in recombination and repair assays were derivatives of AB1157 and were constructed by bacteriophage P1 transduction as described by Miller (34). The identification of Δ*helD*::*cam*, Δ*uvrD*::*tet*, and Δ*recQ*::*kan* mutants among the transductants was accomplished by screening for the appropriate antibiotic resistance followed by cotransduction frequency and Southern blot analysis to confirm the chromosomal deletions.

Chemicals and enzymes. Restriction endonucleases were purchased from New England Biolabs, Inc., and used as specified by the manufacturer. MMS was purchased from Sigma. All other chemicals were of reagent or ultrapure grade.

Media and general methods. Luria-Bertani (LB) media and M56/2 agar and media were prepared as previously described (33). Medium was supplemented when required with tetracycline (7 μg/ml), chloramphenicol (25 μg/ml), kanamycin (50 μg/ml), and appropriate amino acids (40 μg/ml). All other supplements and antibiotics were used at the concentrations recommended by Sambrook et al. (39). UV survival experiments were performed as previously described (33), except that stationary-phase cultures were diluted and spread on LB agar media with the appropriate antibiotics. The plates were then irradiated with UV light (254 nm) for various time intervals, and colonies were counted after a 24- to 48-h incubation in the dark. MMS survival assays and conjugation experiments for determining recombination proficiency were performed as described previously (33). Recombination assays were performed with JC158 (point of origin, PO 1 of Hfr Hayes) as the donor strain. All incubations were at 37°C. Matings were interrupted by vigorous vortexing followed by selection on M56/2

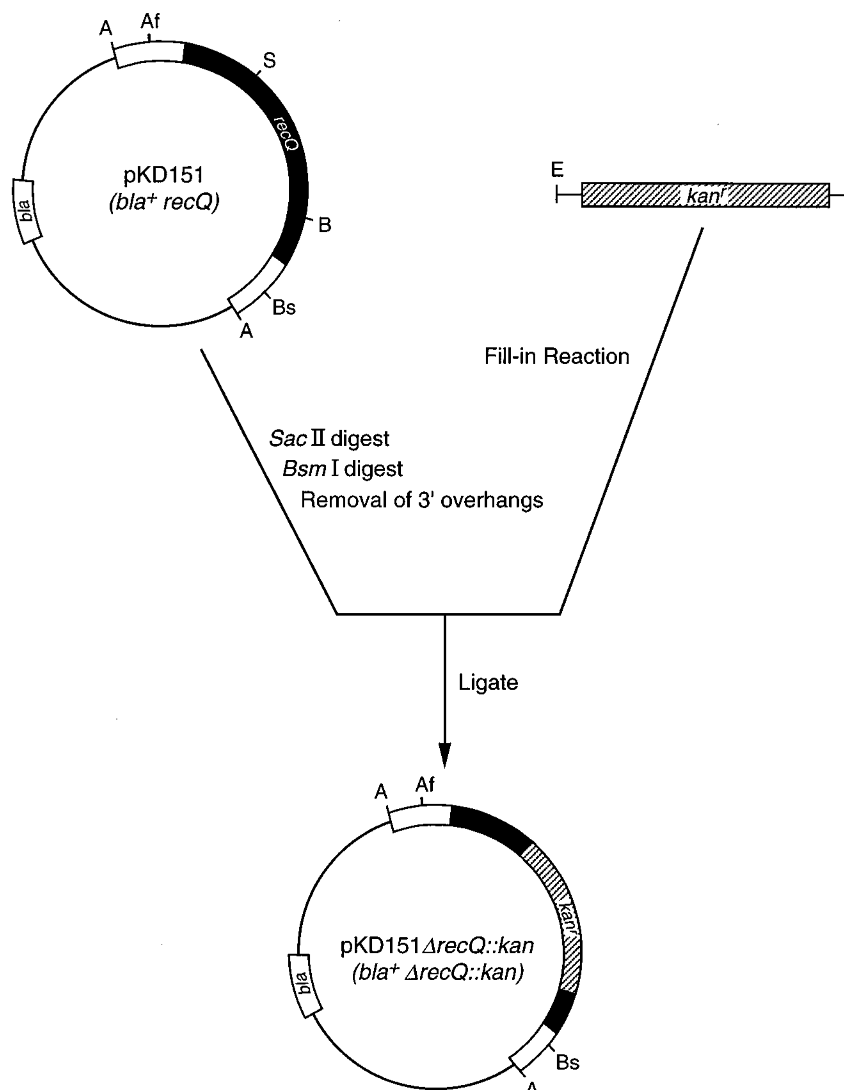

FIG. 1. Construction of pKD151Δ*recQ*::*kan*. See Materials and Methods for details of the construction. Restriction endonuclease sites: A, *Alu*II; Af, *Afl*II; B, *Bsm*I; Bs, *Bst*EII; E, *Eco*RI; S, *Sac*II. Solid boxes, *recQ* coding sequence; open boxes, 3′ and 5′ flanking sequences; hatched boxes, Kan^r gene.

agar media with the appropriate supplements. Transconjugant selection was for leucine and threonine prototrophy. These two markers are transferred early relative to the helicase genes. Counterselection was for serine prototrophy and antibiotic resistance, further ensuring that the wild-type helicase genes are not selected in the transconjugant population. The yield of transconjugants obtained for each strain is expressed as a ratio relative to AB1157, the *rec*$^+$ strain, mated in parallel.

DNA techniques. Plasmids were grown in *E. coli* C600 or HB101. Plasmid DNA was prepared by the alkaline-sodium dodecyl sulfate lysis procedure as described by Sambrook et al. (39) followed by banding in CsCl-ethidium bromide gradients. *E. coli* chromosomal DNA was prepared as described elsewhere (47). DNA restriction fragment probes were radioactively labelled by using the Random Primed DNA Labelling Kit (U.S. Biochemicals) and [α-^{32}P]dCTP according to the manufacturer's specifications. Southern blotting was performed as described by Sambrook et al. (39), using GeneScreen nylon membranes.

Construction of pKD151Δ*recQ*::*kan*. pKD151, a pUC8-based plasmid with an intact copy of the *recQ* gene, was kindly provided by H. Nakayama (46). pKD151 DNA was digested to completion by using *Bsm*I and *Sac*II to remove 466 bp of the coding sequence of the *recQ* gene. The resulting 4.7-kb DNA fragment was isolated and the 3′ overhangs were removed by using T4 DNA polymerase to form blunt ends. A 1.28-kb *Eco*RI DNA fragment containing the kanamycin resistance gene (Kan^r) (Pharmacia) was filled in by using *E. coli* DNA polymerase I (large fragment) and deoxynucleoside triphosphates to form blunt ends. The two blunt-ended DNA fragments were then ligated with T4 DNA ligase. The resulting plasmid, pKD151Δ*recQ*::*kan*, contains a deleted *recQ* gene with an internal Kan^r gene insertion. In addition to the Kan^r gene, it also contains 260 bp of the upstream *recQ* flanking sequence and 318 bp of the downstream *recQ* flanking sequence (Fig. 1). The *Afl*II and *Bst*EII restriction sites present in the *recQ* flanking region of pKD151Δ*recQ*::*kan* were used to produce the 3.22-kb DNA fragment utilized to effect gene replacement in *E. coli* JC7623 (see below).

RESULTS

Construction of a *recQ* deletion-insertion mutant. A *recQ* deletion-insertion mutant strain was constructed by the direct gene replacement technique described by Jasin and Schimmel (14). *E. coli* JC7623 was transformed with a linear *Afl*II-*Bst*EII DNA fragment isolated from pKD151Δ*recQ*::*kan* (Fig. 1). Within this linear DNA fragment a portion of the *recQ* gene has been replaced with a kanamycin resistance gene. A double recombination event, involving DNA sequences present within the transformed DNA fragment containing the Δ*recQ*::*kan* gene and the homologous chromosomal *recQ* gene, results in direct gene replacement.

Potential Δ*recQ* strains were isolated after transformation by selecting for kanamycin-resistant colonies. Direct confirmation

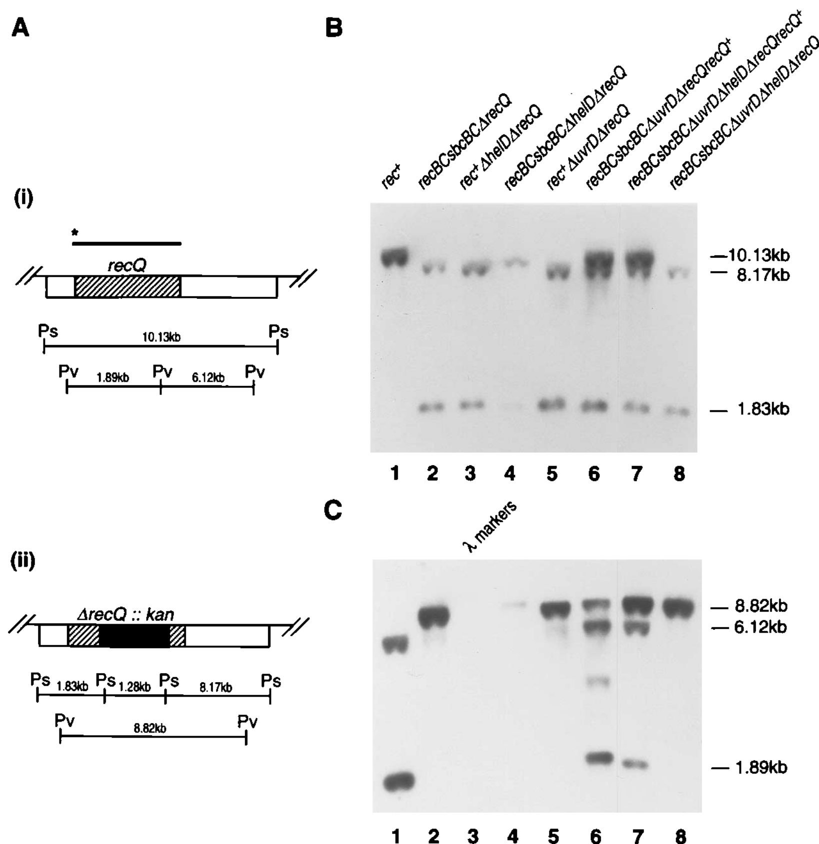

FIG. 2. Southern blot analysis of Δ*recQ* strains. (A) Partial physical maps of the *E. coli* chromosome before (i) and after deletion-insertion (ii) of the *recQ* gene. Restriction fragments expected after *Pst*I (Ps) digestion and *Pvu*II (Pv) digestion are shown. Hatched boxes, *recQ* coding sequence; solid box, Kan^r gene; open boxes, 3′ and 5′ flanking sequences. (B and C) Southern blots of chromosomal DNAs digested with *Pst*I and *Pvu*II, respectively, and probed with a 2.4-kb ^{32}P-DNA fragment containing the wild-type *recQ* gene. Lanes 1, AB1157; lanes 2, SWM2003; lane 3 (B), SWM1011; lanes 4, SWM2011; lanes 5, SWM1050; lanes 6, SWM2050(a); lanes 7, SWM2101(a); lanes 8, SWM2101.

that the *recQ* deletion-insertion was present on the chromosome was obtained by cotransduction frequency analysis (data not shown) and Southern blot analysis of chromosomal DNA isolated from deletion mutants. Each isolate was digested with two different restriction enzymes, *Pst*I and *Pvu*II (Fig. 2). Southern blot analysis of DNA isolated from a *recQ*$^+$ strain and digested with *Pst*I yields a 10.3-kb DNA fragment when probed with the wild-type *recQ* gene sequence (Fig. 2B, lane 1). Chromosomal DNA isolated from a Δ*recQ*::*kan* strain and digested with *Pst*I yields 8.17-, 1.83-, and 1.28-kb DNA fragments; only the 8.17- and 1.83-kb fragments hybridize to the *recQ* probe. The 1.28-kb fragment encodes the Kan^r gene and, therefore, does not hybridize to the *recQ* wild-type probe (Fig. 2B, lane 2). Wild-type chromosomal DNA digested with *Pvu*II and probed with the *recQ* DNA sequence yields DNA fragments 6.12 and 1.89 kb in length (Fig. 2C, lane 1). DNA isolated from the Δ*recQ*::*kan* strain yields a DNA fragment of 8.82 kb as a consequence of the elimination of an internal *Pvu*II site (Fig. 2C, lane 2).

Deletion of the *recQ* gene in the *recBC sbcB*(*C*) strain background does not result in lethality, confirming previous studies indicating that RecQ helicase is not essential for cell viability (38). However, elimination of the RecQ helicase might be compensated for by other helicases present within the cell. Obvious candidates for functional compensation include helicase II, encoded by the *uvrD* gene, and helicase IV, encoded by the *helD* gene (32).

In an effort to probe for potential functional redundancy among helicase II, helicase IV, and RecQ helicase, double and triple helicase mutants were constructed by P1 transduction followed by screening for the appropriate antibiotic resistance (Table 1). Final verification of the existence of the desired deletion-insertion mutations in each cell strain was accomplished by Southern blot analysis and cotransduction frequency analysis (Fig. 2 and data not shown). Using P1 transduction, we were able to move the Δ*recQ*::*kan* deletion-insertion into the following AB1157 (*rec*$^+$) derivatives: SWM1001 (Δ*helD*), SWM1002 (Δ*uvrD*), and SWM1010 (Δ*helD* Δ*uvrD*). In addition, the Δ*recQ*::*kan* deletion-insertion was transduced into the following JC7623 [*recBC sbcB*(*C*)] derivatives: SWM2001 (Δ*helD*), SWM2002 (Δ*uvrD*), and SWM2010 (Δ*helD* Δ*uvrD*). The frequency at which Tet^r Kan^r (Δ*uvrD* Δ*recQ*) and Tet^r Kan^r Cam^r (Δ*uvrD* Δ*recQ* Δ*helD*) transductants were obtained in a *recBC sbcBC* background was extremely low compared with the frequency of Kan^r (Δ*recQ*) or Kan^r Cam^r (Δ*recQ* Δ*helD*) transductants, indicating that the formation of Δ*recQ* Δ*uvrD* mutants was a low-frequency event. In addition, we were unable to obtain any transductants when we attempted to move the Δ*uvrD*::*tet* allele into a *recBC sbcB*(*C*) Δ*recQ*::*kan* background. Analysis by Southern blot of the Tet^r Kan^r transductants obtained when Δ*recQ*::*kan* was moved into Δ*uvrD* strains in a *recBC sbcB*(*C*) background revealed that the potential double mutants contained both the wild-type copy and the deletion-insertion copy of the *recQ* gene (Fig. 2B and C,

lanes 6). The potential Δ*recQ* Δ*uvrD* double mutants contained both the 1.83-kb and the 8.17-kb fragments characteristic of the deletion-insertion and the 10.13-kb fragment characteristic of the wild-type gene when analyzed after *Pst*I digestion of chromosomal DNA. Similarly, they contained the 1.89-, 6.12-, and 8.82-kb fragments when analyzed after *Pvu*II digestion of the chromosomal DNA. Presence of both the wild-type and the mutant *recQ* genes was also observed by using another *recQ* allele (*recQ*:Tn*3*) (38) to isolate *recQ uvrD* double mutants in a *recBC sbcB*(*C*) background (data not shown) (16). This indicates that the chromosomal duplication observed is not specific for the Δ*recQ*::*kan* deletion-insertion. In combination, the above data suggest that the introduction of a *recQ* mutation into a *recBC sbcB*(*C*) Δ*uvrD* background is a lethal event and this selects a population of cells which carry a duplication in the *recQ* portion of the chromosome.

Analysis of the Tet^r Kan^r Cam^r transductants obtained on introduction of Δ*recQ* into a *recBC sbcB*(*C*) Δ*helD* Δ*uvrD* cell strain revealed that seven of the eight transductants analyzed by Southern blot also contained both the wild-type copy and the deleted copy of the *recQ* gene (Fig. 2B and C, lanes 7). A single transductant, however, contained just the deletion-insertion copy of the *recQ* gene (Fig. 2B and C, lanes 8). Helicase II is known to be involved in the methyl-directed mismatch repair pathway of *E. coli*, and consequently, *uvrD* mutants display high mutator frequencies (35). Thus, it is possible that the triple helicase mutant carrying a single copy of the *recQ* deletion-insertion gene may have fortuitously accumulated suppressor mutations rendering it viable. Alternatively, eliminating *helD* may enhance the possibility of obtaining a Δ*recQ* Δ*uvrD* double mutant (with just a single copy of the *recQ* gene) by some unknown mechanism.

Effect of helicase mutations on recombination efficiency. Genetic studies have demonstrated the involvement of RecQ, helicase II, and helicase IV in both homologous recombination and recombinational repair pathways (33, 37). The fact that single null mutations in each of these helicase genes do not exhibit an extreme phenotype with regard to recombination and/or DNA repair may be due to functional overlap between the helicases (25, 33, 37). Previous studies have shown a functional overlap between helicases II and IV in a *recBC sbcB*(*C*) background (33). To determine if there were any synergistic interaction between helicase II/RecQ and helicase IV/RecQ, the deletion-insertion mutants were analyzed for their recombination and repair efficiency. We followed Hfr-mediated conjugational recombination to measure recombination frequency. Introduction of the double Δ*recQ* Δ*helD* and Δ*recQ* Δ*uvrD* and triple Δ*recQ* Δ*helD* Δ*uvrD* helicase mutations in a *rec*$^+$ background did not affect recombination proficiency significantly (Table 2). The recombination efficiencies were similar to that observed for a Δ*helD* Δ*uvrD* double mutant in a *rec*$^+$ background (33). This substantiates previous studies suggesting that RecBCD helicase is the primary helicase involved in conjugational recombination in wild-type *E. coli* (6, 12, 31).

However, analysis of the single, double, and triple helicase mutants in the *recBC sbcB*(*C*) background yielded substantially different results (Table 2). The single *recQ* deletion-insertion mutant showed a 100-fold decrease in recombination efficiency compared with the parent strain JC7623. This decreased efficiency of conjugational recombination was similar to that previously observed for *recQ* insertion mutants in a *recBC sbcB*(*C*) background (37). The Δ*helD* Δ*recQ* double mutant demonstrated recombination efficiency similar to that of the single Δ*recQ* null strain. This suggests that there is no functional redundancy between helicases IV and RecQ. The double Δ*recQ* Δ*uvrD* and the triple Δ*recQ* Δ*uvrD* Δ*helD* null mutants,

TABLE 2. Effect of helicase (*helD*, *uvrD*, and *recQ*) mutations on conjugational recombination[a]

Strain	Relevant genotype	Relative viability	Relative yield of Thr$^+$ Leu$^+$ transconjugants	Recombination efficiency[b]
AB1157	*rec*$^+$	1.0	1.00	1.00
AB1157 derivatives				
SWM1001	Δ*helD*	1.53	2.23	1.46
SWM1002	Δ*uvrD*	0.46	0.14	0.30
SWM1003	Δ*recQ*	0.58	0.58	0.99
SWM1010	Δ*helD* Δ*uvrD*	0.62	0.20	0.32
SWM1011	Δ*helD* Δ*recQ*	1.44	0.89	0.61
SWM1050	Δ*uvrD* Δ*recQ*	1.86	0.30	0.16
SWM1101	Δ*uvrD* Δ*helD* Δ*recQ*	1.43	0.30	0.21
JC7623	*recBC sbcB*(*C*)	0.61	0.55	0.90
JC7623 derivatives				
SWM2001	Δ*helD*	0.75	0.42	0.56
SWM2002	Δ*uvrD*	0.22	0.07	0.32
SWM2003	Δ*recQ*	0.51	0.007	0.01
SWM2010	Δ*helD* Δ*uvrD*	0.45	0.002	0.005
SWM2011	Δ*helD* Δ*recQ*	0.98	0.056	0.06
SWM2101	Δ*uvrD* Δ*helD* Δ*recQ*	0.11	0.00006	0.00055

[a] Matings were done in LB medium at 37°C for 60 min with Hfr JC158 (see Materials and Methods). The values for viability and transconjugants are relative to those for AB1157 strains mated in parallel (see Materials and Methods) and are the means of at least four to six independent sets of experiments. The standard deviations ranged from 5 to 30% of the mean and are excluded here for clarity of presentation. The actual values determined for AB1157 were 2.4×10^8 viable cells and 2.0×10^7 transconjugants per mating mixture.

[b] Ratio of the relative yield of transconjugants to relative viability, included to normalize the differences in viability.

which possessed both a wild-type copy and a deletion-insertion copy of the *recQ* gene, also exhibited recombination frequencies similar to that of a single Δ*recQ* null mutant (data not shown). The triple (Δ*recQ* Δ*uvrD* Δ*helD*) helicase mutant containing the *recQ* deletion-insertion mutation (i.e., lacking a wild-type copy of *recQ*), however, demonstrated significantly reduced recombination efficiency. This decreased recombination frequency is not due to the failure of the triple helicase mutant to support conjugation, as this strain can receive and support an F′ factor (data not shown). The recombination frequency in the triple mutant is similar to that observed for a *recA* null allele (12), indicating an absolute requirement for either RecQ, helicase II, or helicase IV in the RecF pathway of conjugational recombination. The inability to obtain a Δ*recQ* Δ*uvrD* double mutant suggests that the absolute requirement may be for helicase II and RecQ. The absence of helicase IV may be incidental. If the triple helicase mutant had acquired a suppressor mutation (to allow for viability of the Δ*recQ* Δ*uvrD* deletion), its presence did not seem to alleviate the decreased recombination frequency, suggesting the possibility of RecQ or helicase II involvement in an alternate process essential for cell viability.

Effect of helicase mutations on DNA repair. Most of the DNA damage following UV irradiation is eliminated by excision repair enzymes. Some of the damage, which escapes excision repair and undergoes DNA replication, results in the formation of DNA daughter strand gaps and double-strand breaks, most of which are repaired by enzymes catalyzing homologous recombination (41). Helicase II functions in the nucleotide excision repair pathway and is directly responsible for

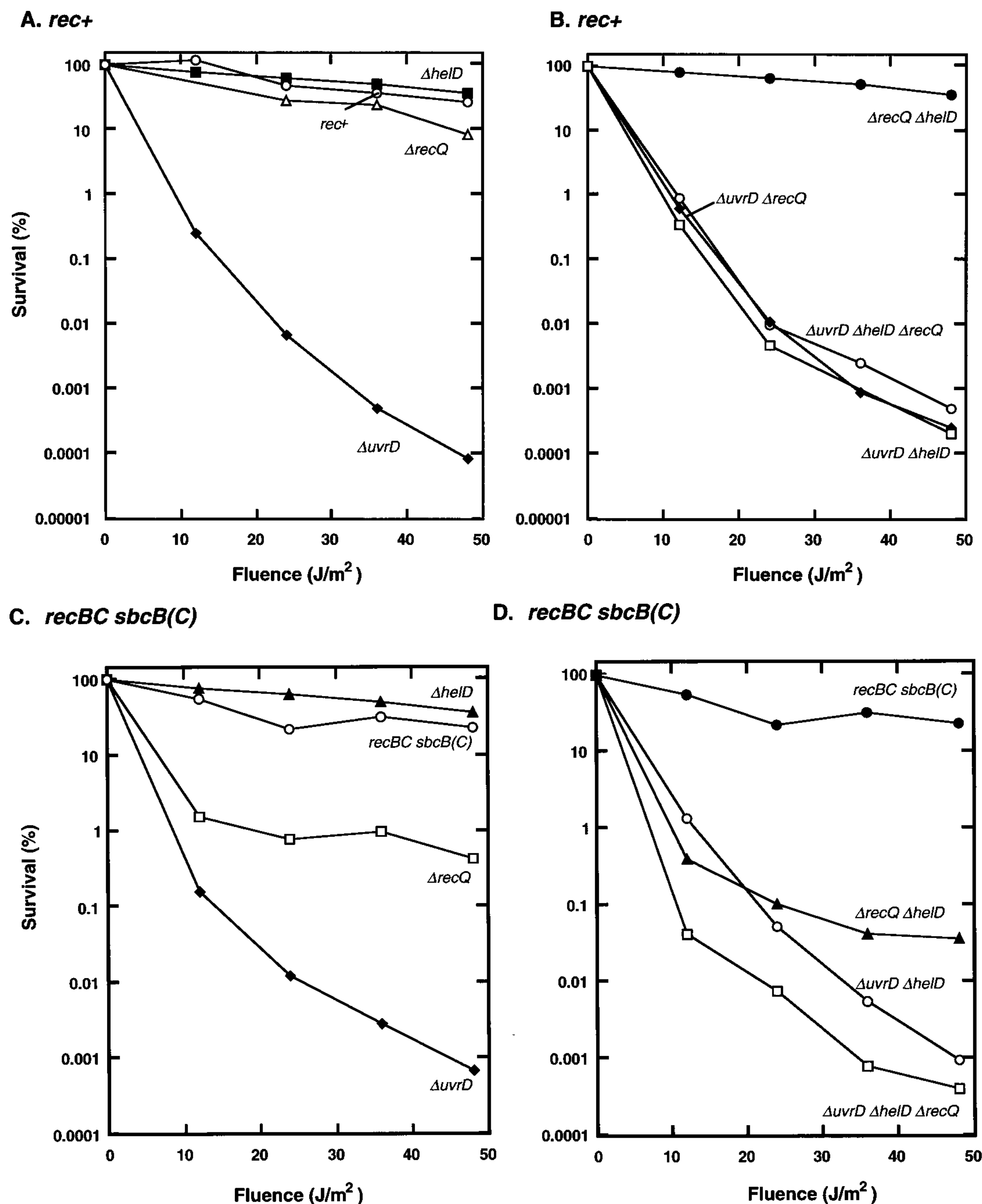

FIG. 3. UV sensitivity of strains carrying mutations in helicase (*helD*, *uvrD*, and *recQ*) genes. Stationary-phase cells plated on LB media were exposed to UV light (254 nm) at the indicated fluence and were incubated at 37°C for 24 h in the dark as described in Materials and Methods. (A) ○, AB1157; ■, SWM1001; △, SWM1003; ◆, SWM1002. (B) ●, SWM1011; ❑, SWM1010; ◆, SWM1050; ○, SWM1101. (C) ○, JC7623; ▲, SWM2001; ❑, SWM2003; ◆, SWM2002. (D) ●, JC7623; ▲, SWM2011; ○, SWM2010; ❑, SWM2101. The data are averages for at least four independent experiments.

release of UvrC from the incision complex following dyad incision on either side of the DNA adduct (41). Single *recQ* and *helD* mutants displayed UV resistance similar to that of a wild-type cell strain (Fig. 3A) (33, 37). The UV survival curves for single, double, and triple helicase mutants in a wild-type rec^+ background are shown in Fig. 3. The Δ*recQ* and Δ*recQ* Δ*helD* strains were as resistant to UV damage as the wild-type rec^+ strain (Fig. 3A and B), demonstrating a lack of synergism in UV repair between helicases IV and RecQ. The Δ*uvrD* Δ*recQ* and Δ*uvrD* Δ*recQ* Δ*helD* strains displayed UV sensitivity similar to that in a single Δ*uvrD* null strain (Fig. 3A). Thus, introduction of a *recQ* mutation in a helicase II-deficient background does not increase the UV sensitivity, suggesting that there is no compensation for helicase II by RecQ helicase with regard to the repair of damaged DNA induced by UV irradiation.

Analysis of the UV sensitivity of the helicase mutants in a *recBC sbcB*(*C*) background detected a synergistic interaction between helicase IV and RecQ. The double mutant displayed an increased UV sensitivity compared with either of the single

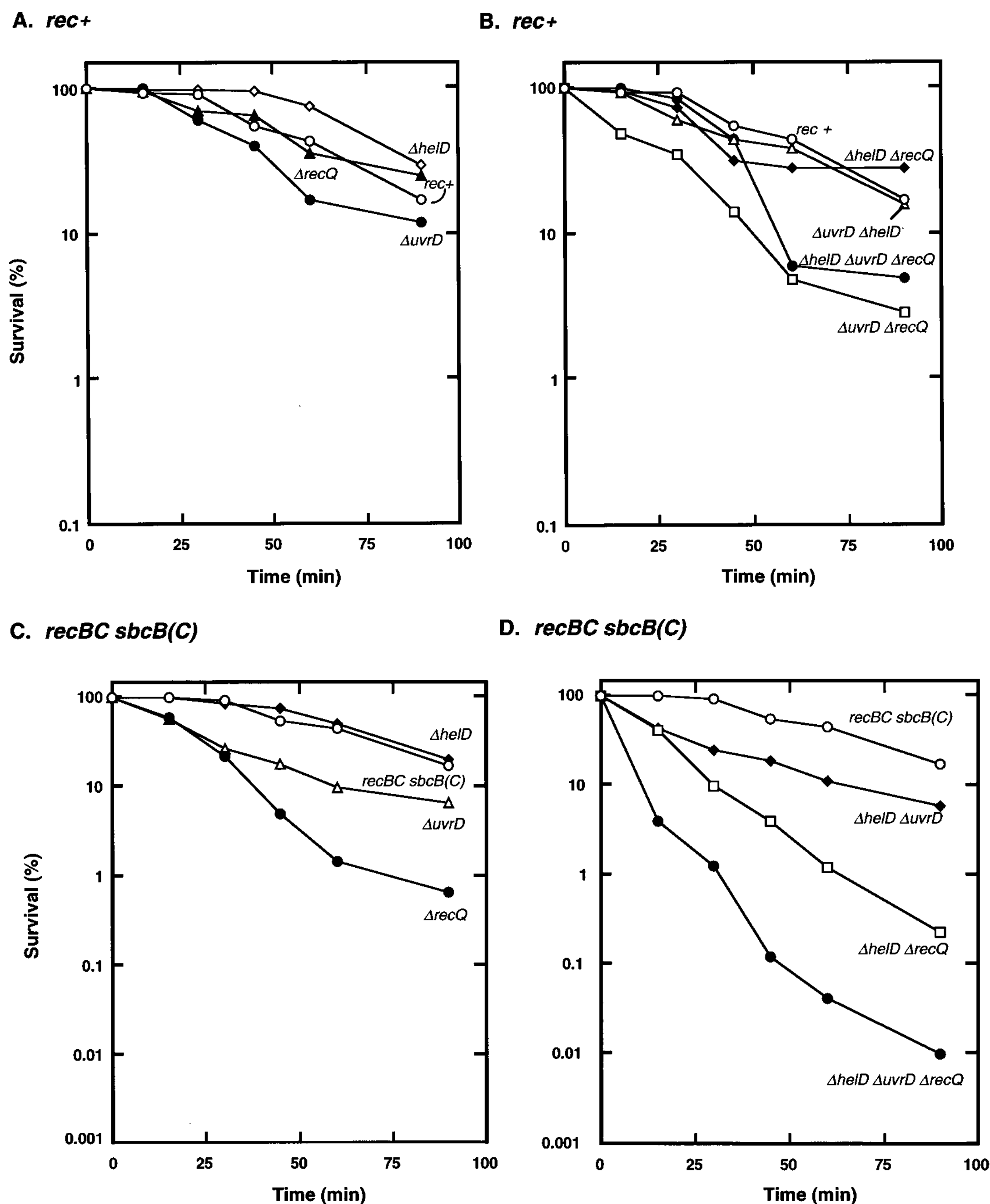

FIG. 4. MMS sensitivity of strains carrying mutations in helicase (*helD*, *uvrD*, and *recQ*) genes. Stationary-phase cells were exposed to 24 mM MMS for the periods indicated and then plated on LB agar with the required antibiotics. (A) ○, AB1157; ◇, SWM1001; ●, SWM1002; ▲, SWM1003. (B) ○, AB1157; ◆, SWM1011; △, SWM1010; ❑, SWM1050; ●, SWM1101. (C) ○, JC7623; ◆, SWM2001; △, SWM2002; ●, SWM2003. (D) ○, JC7623; ◆, SWM2010; ❑, SWM2011; ●, SWM2101. The data are averages for at least four independent experiments.

mutants (Fig. 3C and D) (33, 37). The triple helicase mutant containing the *recQ* deletion-insertion mutation (i.e., no wild-type *recQ* gene) displayed a UV sensitivity similar to that of the single helicase II mutant (Fig. 3), suggesting that the direct involvement of helicase II in the excision repair pathway may mask potential interactions between the helicases with regard to recombinational repair.

We also measured the sensitivity of the helicase mutants to the mutagen MMS (Fig. 4). MMS, like several other alkylating agents, modifies DNA bases, resulting in toxic lesions (10). Removal of the alkylated bases results in the formation of a mutagenic apurinic or apyrimidinic site (10). Helicase II has previously been shown to be required for the efficient repair of DNA damage caused by alkylating agents (33, 40). Helicase IV, on the other hand, has no direct involvement in the repair of MMS-induced damage (33). The single *recQ* mutant exhibited an MMS resistance similar to that of the wild-type AB1157 strain in a rec^+ background (Fig. 4A). The double Δ*recQ* Δ*helD* mutant also displayed an MMS resistance similar to that of AB1157 (rec^+), suggesting absence of synergism between he-

licase IV and RecQ in the repair of MMS-induced DNA damage (Fig. 4B). This substantiates the earlier result of UV repair in which a similar lack of synergism between helicase RecQ and helicase IV in a *rec*$^+$ background was observed. In a wild-type *rec*$^+$ background the double Δ*recQ* Δ*uvrD* mutant and the triple Δ*recQ* Δ*helD* Δ*uvrD* mutant demonstrated increased sensitivity to MMS, greater than that observed for the single helicase mutants (Fig. 4B). This suggests that both helicase II and RecQ are involved in the repair of MMS-damaged DNA.

In a *recBC sbcB*(*C*) background, a Δ*recQ* mutant showed a sensitivity to MMS greater than that of a single Δ*uvrD* mutant (Fig. 4C). The functional redundancy between Δ*recQ* and Δ*helD* for UV-damaged DNA repair was not observed in the case of MMS-damaged DNA repair (Fig. 4D). The triple helicase mutant containing the deletion-insertion mutation of the *recQ* gene (i.e., no wild-type copy of the gene) demonstrated MMS sensitivity that was an order of magnitude greater than that of the most sensitive double mutant (Fig. 4D). Either this increased MMS sensitivity could be due to a redundancy in function between helicase II and RecQ or it could be the result of a synergistic interaction between the three helicases.

DISCUSSION

To ascertain whether synergistic associations between the DNA helicases implicated in the RecF pathway of recombination exist, we have constructed single, double, and triple deletion-insertion mutations in helicases II, IV, and RecQ. These mutants have been analyzed for conjugational recombination efficiency and repair proficiency. In the process of constructing the deletion strains, we discovered that a Δ*recQ* Δ*uvrD* double mutation is apparently lethal in a *recBC sbcB*(*C*) background; however, this has not been rigorously proved. Synthetic lethality could be proved by constructing Δ*recQ* Δ*uvrD* mutants in a *recB*(Ts) *recC*(Ts) background (20) and monitoring viability at the permissive and restrictive temperatures. The apparent synthetic lethality was observed irrespective of the *recQ* allele used; however, we have not attempted to construct the Δ*uvrD* Δ*recQ* double mutation with a different *uvrD* allele. Previous studies have shown that Δ*polA* Δ*uvrD* and Δ*rep* Δ*uvrD* double mutants are inviable or require the presence of suppressors for viability (2, 44). Our inability to construct a viable Δ*recQ* Δ*uvrD* double mutant suggests that eliminating RecQ and helicase II eliminates some critical cellular pathway in a *recBC sbcB*(*C*) background. Both the *uvrD* gene and the *recQ* gene are SOS genes regulated by the product of *lexA* (32, 38). In a *recBC sbcB*(*C*) background they may participate in a damage-inducible process essential for cell viability. Alternatively, in a *recBC sbcB*(*C*) mutant a slight derepression of the *lexA* regulon is observed (15), indicating a persistent low level of DNA damage. Helicase II and RecQ may act synergistically to repair this damage. As yet, we have no evidence to support either of these models and do not understand the reason for the synthetic lethality of the Δ*recQ* Δ*uvrD* double mutant in a RecF background.

We were, however, able to construct a triple deletion mutant of helicases II, IV, and RecQ in the *recBC sbcB*(*C*) background. This may be related to the fact that helicase II is involved in methyl-directed mismatch repair and *uvrD* mutants exhibit an increased mutator frequency (35). The lethality of a Δ*recQ* Δ*uvrD* double mutation may select for the accumulation of suppressor mutations one of which was fortuitously identified among the triple mutants. The existence of a suppressor(s) in the Δ*recQ* Δ*uvrD* Δ*helD* triple mutant has yet to be experimentally verified. Alternatively, elimination of helicase IV may enable construction of the Δ*recQ* Δ*uvrD* mutant. Perhaps helicase IV, in the absence of recQ and helicase II, is involved in an error-prone pathway that is lethal to the cell. If this were the case, then eliminating helicase IV should decrease the mutator frequency of the Δ*recQ* Δ*uvrD* cell strain. However, we have been unable to construct a Δ*recQ* Δ*uvrD* strain and, therefore, cannot compare its mutator frequency to that of the Δ*recQ* Δ*uvrD* Δ*helD* triple mutant.

Previous studies have demonstrated that the RecBCD helicase plays a significant role in conjugational recombination in a wild-type cell (6). Our analysis of the triple helicase deletions in a wild-type background has verified these earlier studies. We were unable to detect any synergistic associations between helicases II, IV, and RecQ with regard to Hfr-mediated recombination proficiency in a wild-type cell strain. Investigating the effect of the helicase mutations in a *recBC sbcB*(*C*) background, we found an absolute requirement for at least one of the three helicases in Hfr-mediated recombination. The triple helicase mutant exhibited recombination deficiencies similar to that observed for a *recA* null allele (12). The deficiency in the triple mutant may be due to the absence of helicases II and RecQ. This could not be tested directly, as we were unable to construct a double Δ*recQ* Δ*uvrD* mutant. Alternatively, it could be a result of the elimination of all three helicases. We would suggest that the severe recombination deficiency is due to the absence of the three helicases for the following reason. In a *recBC sbcB*(*C*) background a Δ*uvrD* Δ*helD* mutant exhibits a 100-fold recombination deficiency compared with the parent. The residual recombination in the double mutant probably proceeds via RecQ protein-initiated substrates (33). Hence, eliminating RecQ in the double Δ*uvrD* Δ*helD* background eliminates the residual recombination. This interpretation assumes that helicase II or IV or the RecQ helicase is compensating for the RecBCD enzyme in a *recBC sbcB*(*C*) strain. However, it is formally possible that the *sbcB*(*C*) mutation is responsible for revealing the requirement for helicase II, IV, or RecQ. This has not been tested experimentally.

The double Δ*recQ* Δ*helD* mutant showed a lack of synergism between helicases IV and RecQ, as evidenced by the fact that the observed recombination deficiency was similar to that in a single *recQ* mutant. We have previously shown a functional overlap between helicases II and IV for conjugational recombination in a *recBC sbcB*(*C*) background (33). The lack of synergism between RecQ and helicase IV can be reconciled with the profound deficiency in recombination efficiency in a triple mutant by suggesting that the functional overlap between helicases II and IV occurs in a homologous recombination pathway following conjugation and the synthetic lethality in a Δ*recQ* Δ*uvrD* double mutant is in a recombinational repair pathway or an as yet unknown critical cellular pathway. An alternate explanation for the lack of synergism between helicases IV and RecQ is that there may be more than one RecF pathway, with helicases IV and II involved in one pathway and RecQ involved in the other. These results also highlight an important observation made in most of the recent genetic studies (6, 17, 25, 29, 43): recombination mechanisms depend both on the initiating DNA substrates and on the gene products present in the cell at any given time.

Though the course of recombination seems to be dictated by the DNA substrate presented, most of the genes involved in recombination have been shown to have a significant role in DNA repair (8, 12, 23, 26, 27, 37). It has been proposed that homologous recombination has evolved because it is beneficial for repair (4). One of the striking features of the RecF pathway genes is that these mutations have a broad variety of effects on DNA repair in a *rec*$^+$ background while having no effect on

Hfr-mediated recombination proficiency (17). For example, mutations in the *recF*, *recN*, *ruv*, and *recQ* genes of the RecF pathway have no effect on conjugational recombination but, with the exception of *recQ*, do have an effect on DNA repair (23, 26, 27, 37). One of the reasons for the absence of an effect on DNA repair in a *recQ* mutant may be functional compensation by the *uvrD*-encoded helicase II. This compensation is unmasked in a Δ*recQ* Δ*uvrD* double mutant for which we observed a functional overlap between helicases II and RecQ with regard to repair of MMS-damaged DNA in a rec^+ background.

Analyzing the effect of the helicase mutants in a *recBC sbcB*(*C*) background in terms of DNA repair detected a direct role for RecQ helicase in repair of MMS-induced DNA damage. Previous evidence for the role of RecQ in DNA repair includes an increased sensitivity to H_2O_2 and gamma ray and UV irradiation in a *recBC sbcA* background (19) and increased sensitivity to UV irradiation in a *recBC sbcB*(*C*) background (37). The double Δ*helD* Δ*recQ* mutant unmasks the functional redundancy between helicases IV and RecQ with regard to repair of UV-damaged DNA. Hence, depending on the type of DNA damage, helicase IV may or may not be able to partially compensate for RecQ helicase.

The results presented above suggest the following. (i) The genetics of recombination is governed by the genotype of the cell and the structure of the initiating DNA substrates. This can be detected by the different phenotypes and varying ranges of sensitivity exhibited by the helicase mutants in different genetic backgrounds when exposed to different DNA-damaging treatments. (ii) There is functional overlap between helicases II, IV, and RecQ. These synergistic associations are unmasked only in double and triple mutants assayed for repair and recombination deficiency in specific genetic backgrounds. (iii) In addition to DNA recombination, helicase II and RecQ helicase may be involved in an alternate process essential for cell viability.

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