Genetic Analysis of Δ*helD* and Δ*uvrD* Mutations in Combination with Other Genes in the RecF Recombination Pathway in *Escherichia coli*: Suppression of a *ruvB* Mutation by a *uvrD* Deletion

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ABSTRACT

Helicase II (*uvrD* gene product) and helicase IV (*helD* gene product) have been shown previously to be involved in the RecF pathway of recombination. To better understand the role of these two proteins in homologous recombination in the RecF pathway [*recBCsbcB(C)* background], we investigated the interactions between *helD*, *uvrD* and the following RecF pathway genes: *recF*, *recO*, *recN* and *ruvAB*. We observed synergistic interactions between *uvrD* and the *recF*, *recN*, *recO* and *recG* genes in both conjugational recombination and the repair of methylmethane sulfonate (MMS)-induced DNA damage. No synergistic interactions were detected between *helD* and the *recF*, *recO* and *recN* genes when conjugational recombination was analyzed. We did, however, detect synergistic interactions between *helD* and *recF/recO* in recombinational repair. Suprisingly, the *uvrD* deletion completely suppressed the phenotype of a *ruvB* mutation in a *recBCsbcB(C)* background. Both conjugational recombination efficiency and MMS-damaged DNA repair proficiency returned to wild-type levels in the Δ*uvrDruvB9* double mutant. Suppression of the effects of the *ruvB* mutation by a *uvrD* deletion was dependent on the *recG* and *recN* genes and not dependent on the *recF/O/R* genes. These data are discussed in the context of two "RecF" homologous recombination pathways operating in a *recBCsbcB(C)* strain background.

HOMOLOGOUS recombination is thought to proceed by a series of enzymatically catalyzed reactions acting sequentially to convert substrates (parental DNA) into products (recombinant DNA). This view led CLARK to postulate the existence of pathways of recombination in *Escherichia coli* (CLARK 1973) and, subsequently, to the identification of three distinct pathways of homologous recombination: the RecBCD pathway, RecF pathway and the RecE pathway. However, subsequent genetic studies have revealed the limitations of mutually exclusive pathways, and it has become evident that the course of recombination for a particular DNA substrate depends on both the structure of the substrate molecule and the gene products available in the cell at the time of recombination (KOLODNER *et. al.* 1985; LLOYD and SHARPLES 1992). For example, recent genetic studies indicate that the genes previously classified together in the RecF pathway of recombination do not form a single homogenous group, and it has been suggested that there may be two "RecF" pathways. These pathways have been tentatively called the RecN and the RecFOR pathways (CLARK 1991).

The *recF*, *recO* and *recR* gene products (RecF, RecO and RecR, respectively) have been linked via genetic and biochemical studies and apparently function as a protein complex in a presynaptic stage of recombination (CLARK 1991; LLOYD and SHARPLES 1992; UMEZU *et al.* 1993; SANDLER and CLARK 1994). Biochemical studies suggest that these proteins may aid RecA protein in the efficient use of single-stranded DNA binding protein-coated DNA as a recombinogenic substrate (UMEZU *et. al.* 1993; SANDLER and CLARK 1994). The *recN* gene has been placed in a different epistasis group because its mutant allele exhibits synergistic interactions with *recF*, *recO* and *recR* (LLOYD and BUCKMAN 1991). In addition, *recN*, unlike *recF*, *recO* and *recR*, does not affect the formation of transcribable intermediates in *recB* strains (LLOYD *et al.* 1987) or plasmid recombination efficiency in *recBC*$^+$ *sbcBC*$^+$ strains (KOLODNER *et al.* 1985). The RecN protein has yet to be assigned a biochemical function. However, genetic studies have suggested it to be a functional equivalent of the RecJ nuclease (LLOYD and BUCKMAN 1991). The *ruvB* and *recQ* genes have been placed in the *recN* epistasis group based on a common *lexA* regulation, and the fact that the *recQ* and *recN* genes display synergistic interactions with the *recF* gene (CLARK 1991; LLOYD and BUCKMAN 1991). The *ruvB* gene encodes the RuvB ATPase, which, together with the RuvA protein, forms the RuvAB helicase capable of mediating branch-migration of Holliday junctions (TSANEVA *et al.* 1993).

The *recQ* gene encodes a 3′ to 5′ DNA helicase (UMEZU *et al.* 1990). In addition to the RecQ helicase,

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TABLE 1

Bacterial strains

Strain designation	Relevant genotype	Source
AB1157	*rec*$^{+}$ [a]	HOWARD-FLANDERS *et al.* (1966)
JC158	*lacI22 l*$^{-}$ *serA6 spoT1 thi-1*	CLARK and MARGULIES (1965)
AB1157 Derivatives		
TNM1072	*recG263*	MANDAL *et al.* (1993)
JC7623	*recB21 recC22 sbcB15 sbcC201*	COHEN and CLARK (1986)
JC7623 Derivatives		
JC8111	*recF143*	HORII and CLARK (1973)
JC18924	*recF400::*Tn5	SANDLER and CLARK (1994)
SWM2001	Δ*helD::cam*	This study
SWM2002	Δ*uvrD::tet*	This study
N2730	*recG258::kan*	RYDER *et al.* (1994)
SWM2004	Δ*recG263*	P1.TNM1072 × JC7623 to Kan^{r}
RDK1530	*recN1502::*Tn5	KOLODNER *et al.* (1985)
RDK1531	*recO1504::*Tn5	KOLODNER *et al.* (1985)
RDK1645	*ruvB9*	LUISI-DELUCA *et al.* (1989)
SWM2300	*ruvB9 recF*	P1.JC18924 × RDK1645 to Kan^{r}
SWM2301	*ruvB9* Δ*recG263*	P1.TNM1072 × RDK1645 to Kan^{r}
SWM2012	*recF143* Δ*helD*	P1.SWM2001 × JC8111 to Cam^{r}
SWM2013	*recN1502* Δ*helD*	P1.SWM2001 × RDK1530 to Cam^{r}
SWM2014	*recO1504* Δ*helD*	P1.SWM2001 × RDK1531 to Cam^{r}
SWM2015	*ruvB9* Δ*helD*	P1.SWM2001 × RDK1645 to Cam^{r}
SWM2051	*recF143* Δ*urvD*	P1.SWM2002 × JC811 to Tet^{r}
SWM2052	*recN1502* Δ*uvrD*	P1.SWM2002 × RDK1530 to Tet^{r}
SWM2053	*recO1504* Δ*uvrD*	P1.SWM2002 × RDK1531 to Tet^{r}
SWM2054	*ruvB9* Δ*uvrD*	P1.SWM2002 × RDK1645 to Tet^{r}
SWM2055	*recG258* Δ*uvrD*	P1.SWM2002 × N2730 to Tet^{r}
SWM2056	Δ*recG263* Δ*uvrD*	P1.TNM1072 × SWM2002 to Kan^{r}
SWM3051	*ruvB9* Δ*uvrD* Δ*recF*	P1.JC18924 × SWM2054 to Kan^{r}
SWM3052	*ruvB9* Δ*uvrD recO1504*	P1.RDK1531 × SWM2054 to Kan^{r}
SWM3053	*ruvB9* Δ*uvrD recN1502*	P1.RDK1530 × SWM2054 to Kan^{r}
SWM3054	*ruvB9* Δ*uvrD recG263*	P1.TNM1072 × SWM2054 to Kan^{r}

[a] *F*$^{-}$ *thr-1 leuB6 thi-1 lacY1 galK2 ara14 xyl5 mtl-1 proA2 his4 argE3 rpsL3* (*Sm*^{r}) *tsx-33 supE44 kdgK51.*

helicase II (*uvrD* gene product) and helicase IV (*helD* gene product), have been implicated in the RecF pathway of recombination (MENDONCA *et al.* 1993). The deletion of either *uvrD* or *helD* has little effect on Hfr-mediated recombination frequency. The double Δ*helD* Δ*uvrD* mutant, however, revealed a synergistic interaction between helicase IV and helicase II in a *recBCsbcB(C)* background (MENDONCA *et al.* 1993). The decrease in recombination frequency observed in the double Δ*helD* Δ*uvrD* mutant could be due to the ability of each helicase to channel the same DNA substrate into one or another enzymatic route (or pathway) to recombinants or direct compensation of one helicase activity for the other (*i.e.*, functional redundancy of the helicases) in a single pathway. Recent genetic studies investigating the interactions between the *recQ*, *uvrD* and the *helD* genes have demonstrated an extreme recombination and repair deficiency in a triple deletion mutant in a *recBCsbcB(C)* background (MENDONCA *et al.* 1995). This suggests that the presence of RecQ helicase, helicase II or helicase IV is required for efficient recombination and repair in a *recBCsbcB(C)* background and supports the notion that multiple helicases are required in mutiple recombination pathways in the cell (ROSENBERG and HASTINGS 1991).

In this paper, we report on the analysis of Δ*helD* and Δ*uvrD* mutations in combination with mutant alleles in the following RecF pathway genes: *recF*, *recN*, *recO* and *ruvB*. The results reveal synergistic interactions between *uvrD* and the *recF*, *recN* and *recO* genes. We also observed an interaction between *helD* and *ruvB*, and the ability of a *uvrD* deletion mutation to completely suppress the phenotype of a *ruvB* mutant. The suppression of *ruvB* by Δ*uvrD* was dependent on the *recG* and *recN* genes. Taken together, the data reported in this paper support the notion of two RecF pathways for conjugational recombination (CLARK 1991; LLOYD and BUCKMAN 1991).

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 (1972). The identification of Δ*helD::cam* and Δ*uvrD::tet* mutants among the transductants was accomplished by selecting for the appropriate

TABLE 2

Effect of Δ*uvrD* and Δ*helD* mutations of Hfr-mediated recombination

Strain	Relevant genotype	Relative viability[a]	Relative yield of Thr$^+$ Leu$^+$ transconjugant[a]
AB1157	*rec*$^+$	1.2	1.3
JC7623	*recBC sbcB(C)*	1.0 = 1.9×10^8	1.0 = 1.64×10^7
JC7623 derivatives			
SWM2001	Δ*helD*	0.93 ± 0.11	0.71 ± 0.36
SWM2002	Δ*uvrD*	0.27 ± 0.03	0.41 ± 0.05
JC8111	*recF143*	0.83 ± 0.09	0.025 ± 0.0018
SWM2012	*recF143* Δ*helD*	1.03 ± 0.06	0.012 ± 0.005
SWM2051	*recF143* Δ*uvrD*	0.48 ± 0.04	0.0011 ± 0.0001
RDK1530	*recN1502*	1.12 ± 0.21	0.010 ± 0.002
SWM2013	*recN1502* Δ*helD*	1.02 ± 0.13	0.011 ± 0.001
SWM2052	*recN1502* Δ*uvrD*	0.52 ± 0.05	0.00051 ± 0.00004
RDK1531	*recO1504*	1.17 ± 0.12	0.0012 ± 0.0005
SWM2014	*recO1504* Δ*helD*	0.98 ± 0.08	0.0013 ± 0.0001
SWM2053	*recO1504* Δ*uvrD*	0.32 ± 0.06	0.00088 ± 0.00004
RDK1645	*ruvB9*	0.83 ± 0.04	0.0037 ± 0.0002
SWM2015	*ruvB9* Δ*helD*	1.42 ± 0.16	0.0011 ± 0.0001
SWM2054	*ruvB9* Δ*uvrD*	0.90 ± 0.11	0.608 ± 0.053

Matings were performed in LB media at 37°C for 60 min with donor Hfr JC158 and the appropriate recipient cultures grown to an A_{600} of 0.4 (~2×10^8 cells/ml as determined by viable count) before mixing. Derivative values are means ± SD.

[a] The values for viability and transconjugants given are relative to JC7623 strains mated in parallel (see MATERIALS AND METHODS) and are the means of at least two to six independent sets of experiments. The values for the control strain JC7623 (set equal to 1.0) are per milliliter of recipient culture (viability) or mating mixture (transconjugants).

antibiotic resistance followed by cotransduction frequency analysis and Southern blot analysis to confirm the chromosomal deletions.

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

Media and general methods: Luria-Bertani media, M56/2 agar and M56/2 media were prepared as previously described (MENDONCA *et al.* 1993) and supplemented, when required, with tetracycline (7 μg/ml), chloramphenicol (25 μg/ml), kanamycin (50 μg/ml) and appropriate amino acids (40 μg/ml). Ultraviolet (UV) irradiation survival experiments were performed as previously described (MENDONCA *et al.* 1993) except that stationary cultures were diluted and spread on LB agar media with the appropriate antibiotics. The plates were then irradiated with UV light (254 nm) for varied time intervals and surviving colonies were counted after a 24–48-hr incubation in the dark. MMS survival assays and conjugation experiments for determining recombination proficiency were performed as described previously (MENDONCA *et al.* 1993). Matings were interrupted by vigorous vortexing followed by selection on M56/2 agar media with the appropriate supplements. Transconjugant selection was for leucine and threonine prototrophy, counter selection was for serine prototrophy and antibiotic resistance. The yield of transconjugants obtained for each strain is expressed as a ratio relative to JC7623, the *recBCsbcB(C)* strain, mated in parallel. *E. coli* chromosomal DNA was prepared as described (WILSON 1989). DNA restriction fragment probes were radioactively labeled using the "Random Primed DNA Labeling Kit" (US Biochemicals) and [α-^{32}P]dCTP according to manufacturers' specifications. Southern blotting was performed as described by SAMBROOK *et al.* (1989) using Genescreen nylon membranes.

RESULTS

Interactions between *helD*, *uvrD* and the RecF pathway genes: In an effort to understand the role of the *helD* and *uvrD* gene products in the RecF pathway of recombination, we investigated genetic interactions between *helD*, *uvrD* and the following RecF pathway genes: *recF*, *recN*, *recO* and *ruvB*. Double mutants carrying either the Δ*uvrD* or the Δ*helD* mutation and a *recF*, *recO*, *recN* or *ruvB* mutation were constructed in *E. coli* JC7623 [a *recBCsbcB(C)* background] (Table 1). Introduction of the Δ*uvrD::tet* (helicase II deletion) and Δ*helD::cam* (helicase IV deletion) mutations into the *recF*, *recO*, *recN* and *ruvB* strains was verified by Southern blot analysis (data not shown). All of the double mutants constructed were viable.

A conjugational recombination assay using JC158 as the Hfr donor strain and the appropriate mutants as the recipients was used to analyze the recombination proficiency of each double mutant. The single *recF*, *recO*, *recN* and *ruvB* mutants exhibited decreased recombination efficiency in the *recBCsbcB(C)* background as expected for genes whose protein products have been shown to be directly involved in the RecF pathway of homologous recombination. Analysis of the conjugational recombination proficiency of the double mutants revealed synergistic interactions between *uvrD* and *recF*, *recO* and *recN*, as evidenced by the substantially reduced recombination frequency in the double mutants as compared with the parental strains (Table 2). The Δ*helD*

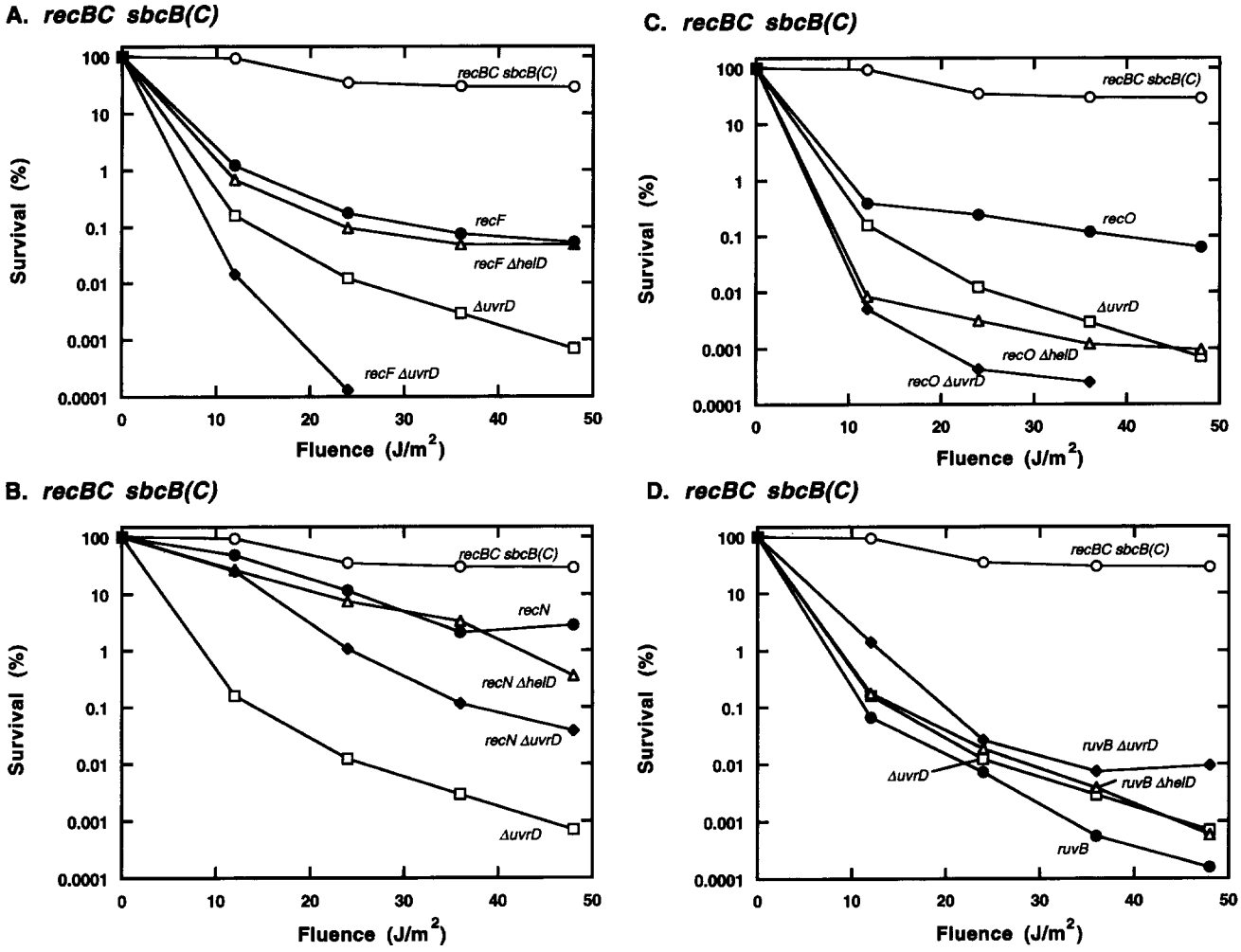

FIGURE 1.—UV sensitivity of strains with mutations in genes involved in the recF pathway of recombination. Stationary cells plated on LB media were exposed to UV light (254 nm) at the indicated fluence, and were subsequently incubated at 37°C for 24 hr in the dark as described under MATERIALS AND METHODS. (A) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, JC8111 (*recF143*); △, SWM2012 (*recF143* Δ*helD*); □, SWM2002 (Δ*uvrD*); ♦, SWM2051 (*recF143* Δ*uvrD*); ▲, SWM2001 (Δ*helD*). (B) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, RDK1530 (*recN1502*); △, SWM2013 (*recN1502* Δ*helD*); □, SWM2002 (Δ*uvrD*); ♦, SWM2052 (*recN1502* Δ*uvrD*); ▲, SWM2001 (Δ*helD*). (C) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, RDK1531 (*recO1504*); □, SWM2002 (Δ*uvrD*); △, SWM2014 (*recO1504* Δ*helD*); ♦, SWM2053 (*recO1504* Δ*uvrD*); ▲, SWM2001 (Δ*helD*). (D) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, RDK1645 (*ruvB9*); □, SWM2002 (Δ*uvrD*); △, SWM2015 (*ruvB9* Δ*helD*); ♦, SWM2054 (*ruvB9* Δ*uvrD*); ▲, SWM2001 (Δ*helD*). The data presented represents the average of at least four independent experiments.

mutation, on the other hand, did not show any significant interactions with regard to conjugational recombination with *recF*, *recO* or *recN* (Table 2). The recombination proficiencies observed in these double mutants was similar to that observed in the *recF*, *recO* and *recN* parental strains (Table 2). However, we did observe an interaction between *helD* and the *ruvB* gene. The Δ*helD ruvB9* mutant exhibited a moderate increase in recombination deficiency as compared with either of the parental strains (Table 2). This could be the result of partial functional compensation of the RuvAB helicase (branch-migrating activity) by helicase IV. *In vitro* biochemical studies have detected the ability of helicase IV to eliminate recombination intermediates (V. M. MENDONCA and S. W. MATSON, unpublished observations).

To verify these results, and to explore further the role of these genes in recombinational repair, UV irradiation and MMS survivorship studies were performed using each mutant strain. The UV and MMS survival curves obtained with the Δ*uvrD recF* and the Δ*uvrD recO* double mutants substantiated the synergistic interactions observed in the conjugational recombination assay (Figures 1, A and C, and 2, A and C). The double mutants displayed UV and MMS sensitivities much greater than observed for either of the single mutants. The Δ*uvrD recN* double mutant also exhibited synergistic interactions between helicase II and the RecN protein. However, these synergistic interactions were only detected for repair of MMS-damaged DNA (Figure 2B). Interestingly, the *recN* mutation suppressed, to

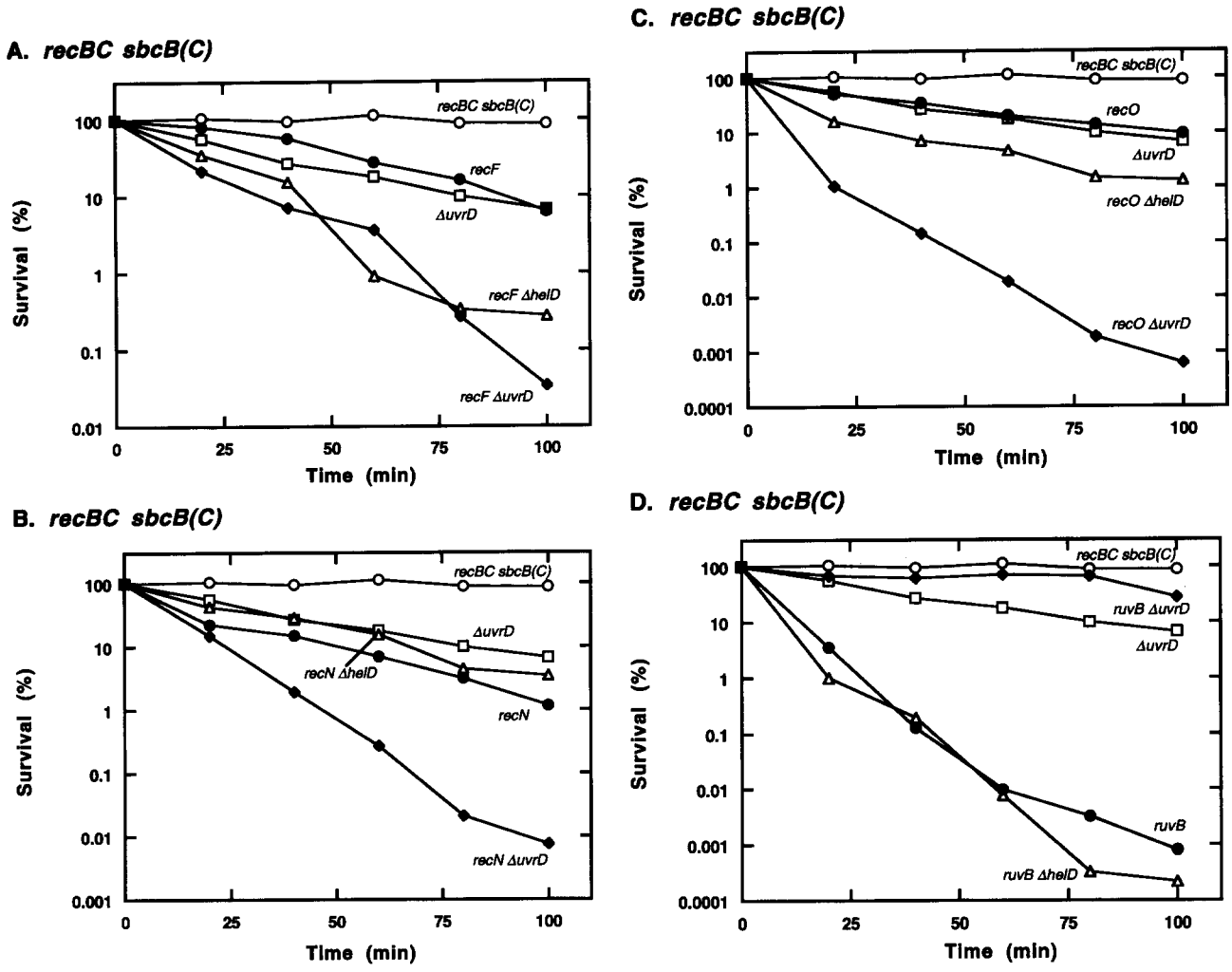

FIGURE 2.—MMS sensitivity of strains with mutations in genes involved in the recF pathway of recombination. Stationary cells were exposed to 24 mM MMS for the time periods indicated and then plated on LB agar with the required antibiotics. (A) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, JC8111 (*recF143*); △, SWM2012 (*recF143* Δ*helD*); □, SWM2002 (Δ*uvrD*); ◆, SWM2051 (*recF143* Δ*uvrD*); ▲, SWM2001 (Δ*helD*). (B) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, RDK1530 (*recN1502*); △, SWM2013 (*recN1502* Δ*helD*); □, SWM2002 (Δ*uvrD*); ◆, SWM2052 (*recN1502* Δ*uvrD*); ▲, SWM2001 (Δ*helD*). (C) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, RDK1531 (*recO1504*); □, SWM2002 (Δ*uvrD*); △, SWM2014 (*recO1504* Δ*helD*); ◆, SWM2053 (*recO1504* Δ*uvrD*); ▲, SWM2001 (Δ*helD*). (D) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, RDK1645 (*ruvB9*); □, SWM2002 (Δ*uvrD*); △, SWM2015 (*ruvB9* Δ*helD*); ◆, SWM2054 (*ruvB9* Δ*uvrD*); ▲, SWM2001 (Δ*helD*). This data represents the average of at least four independent experiments.

some extent, the UV sensitivity of cells with a single Δ*uvrD* mutation (Figure 1B). The significance of this observation is unclear at this time.

Synergistic interactions were also detected between the *helD* gene product and the *recF* and the *recO* gene products in the repair assays (Figures 1, A and C, and 2, A and C). The Δ*helD recO* mutant displayed increased UV and MMS sensitivity as compared with the parental strains. The synergistic interaction between helicase IV and the RecF protein was observed only for the repair of MMS-damaged DNA (Figure 2A). The functional overlap corroborates previous results where we detected a role for helicase IV in the repair of UV-damaged DNA in Δ*recQ* Δ*helD* double mutants (MENDONCA *et al.* 1995). No synergistic interactions were detected in the *recN* Δ*helD* double mutant (Figures 1B and 2B). This result is consistent with the results obtained for this mutant in conjugational recombination assays. However, the apparent functional overlap between the RuvAB helicase and helicase IV detected in conjugational recombination was not evident when we assayed the repair proficiency of the Δ*helD ruvB* mutant (Figures 1D and 2D). The MMS survival curve of the Δ*helD ruvB* double mutant (Figure 2D) was similar to that for the single *ruvB* mutant.

Analysis of Δ*uvrD ruvB9* mutants revealed the unexpected capability of a Δ*uvrD* mutation to completely suppress the effect of the *ruvB* mutation on conjugational recombination (Table 2). The suppression was not specific for conjugational recombination but was also observed when the cells were examined in an MMS survival experiment (Figure 2D). The Δ*uvrD ruvB* mu-

TABLE 3

Effect of *recF*, *recO*, *recN* and *recG* mutations on Δ*uvrD* suppression of a *ruvB* mutation as monitored by transconjugant formation in conjugational crosses

Strain	Relevant genotype	Relative viability[a]	Relative yield of Thr$^+$ Leu$^+$ transconjugants[a]
JC7623	*recBC sbcB(C)*	$1.0 = 4.13 \times 10^8$	$1.0 = 6.7 \times 10^7$
JC7623 Derivatives			
SWM2054	*ruvB9* Δ*uvrD*	0.73 ± 0.09	0.62 ± 0.053
SWM3051	*ruvB9* Δ*uvrD* Δ*recF*	0.61 ± 0.11	0.925 ± 0.059
SWM2300	*ruvB9 recF*	0.27 ± 0.013	0.0011 ± 0.00013
SWM3052	*ruvB9* Δ*uvrD recO1504*	0.65 ± 0.011	0.887 ± 0.072
SWM3053	*ruvB9* Δ*uvrD recN1502*	0.69 ± 0.07	0.025 ± 0.004
SWM3054	*ruvB9* Δ*uvrD recG263*	0.44 ± 0.062	0.005 ± 0.0008
SWM2301	*ruvB9 recG263*	0.20 ± 0.03	0.000075 ± 0.000011

Matings were performed in LB media at 37°C for 60 min with donor Hfr JC158 and the appropriate recipient cultures grown to an A_{600} of 0.4 (~2×10^8 cells per ml as determined by viable count) before mixing. Derivative values are means ± SD.

[a] The values for viability and transconjugants given are relative to JC7623 strains mated in parallel (see MATERIALS AND METHODS) and are the means of at least two to six independent sets of experiments. The values for JC7623 the control strain (set equal to 1) are per milliliter of recipient culture (viability) or mating mixture (transconjugants).

tant displayed a repair proficiency similar to the *uvrD*$^+$ *ruvB*$^+$ parent strain as compared with the MMS-sensitive phenotype observed for the *ruvB* single mutant (Figure 2D). Because helicase II is directly involved in the UvrABC-mediated excision repair pathway (SANCAR and SANCAR 1988; ORREN *et al.* 1992), we did not expect Δ*uvrD* mutants to suppress the UV-sensitive phenotype of *ruv* mutants (Figure 2D). Helicase II plays a direct role in the methyl-directed mismatch repair pathway, and therefore *uvrD* mutants exhibit an increased mutator frequency (KUSHNER *et al.* 1978; ARTHUR and LLOYD 1980). To rule out the possibility of the accumulation of suppressor mutations, several isolates of *recBCsbcB(C)* Δ*uvrD ruvB* mutants were analyzed and alternate *ruvB* alleles were used. In all cases, we observed complete suppression of recombination defects on the introduction of the Δ*uvrD::tet* mutation. In addition, the introduction of a plasmid expressing helicase II in a *recBCsbcB(C) ruvB9* Δ*uvrD* mutant resulted in an increased sensitivity to MMS (data not shown).

Suppression of the *ruvB* phenotype by Δ*uvrD* requires the products of the *recG* and *recN* genes: The suppression of the *ruvB* phenotype by the Δ*uvrD* mutation has at least two possible explanations. (1) Helicase II, in the absence of the RuvAB helicase, might be responsible for eliminating or preventing the extension of Holliday junctions. This would block the completion of a recombination event and decrease recombination efficiency. (2) Helicase II might be responsible for generating recombinogenic ssDNA ends that are then acted upon by RecA (aided by RecF, RecO and RecR), RuvAB and RuvC to form viable recombinants. This latter scenario envisions at least two recombination pathways operating in a *recBCsbcB(C)* background. In one of these pathways, the substrates are generated by helicase II and resolved exclusively via the action of the RuvAB helicase.

In an effort to distinguish between these two possibilities, we introduced additional mutations in the *recBCsbcB(C)ruvB9* Δ*uvrD* mutant and assayed both conjugational recombination and recombinational repair. The rationale for this approach was as follows: if helicase II was responsible for the initial step in the recombination pathway (*i.e.*, generation of recombinogenic ssDNA) then mutations in genes required in subsequent steps should have no effect on the suppression of the *ruvB* phenotype by the Δ*uvrD* mutation. If, however, helicase II acted at the postsynaptic level of recombination (*i.e.*, in branch migration or resolution of Holliday junctions), then mutations in genes whose protein products are required for the presynaptic or synaptic steps in recombination should decrease the proficiency of recombination and DNA repair in the *recBCsbcB(C)-ruvB9* Δ*uvrD* mutant.

The mutations we chose to introduce in the *recBCsbcB(C) ruvB* Δ*uvrD* background were *recF*, *recO*, *recR*, *recN* and *recG*. Analysis of the conjugational recombination proficiency of these mutants showed that suppression of the *ruvB* phenotype by Δ*uvrD* was dependent on the protein products of the *recN* and the *recG* genes (Table 3). Introduction of the *recG* mutation or the *recN* mutation in a *recBCsbcB(C) ruvB* Δ*uvrD* strain resulted in a significant decrease in conjugational recombination efficiency, 10-fold for the *recN* mutant and 100-fold for the *recG* mutant as compared with the *ruvB* Δ*uvrD* strain (Table 3). We also observed a decrease in repair proficiency when either the *recG* or the *recN* mutations were introduced in the *recBCsbcB(C) ruvB* Δ*uvrD* strain. Nevertheless, the recombination and repair proficiency of the *recBCsbcB(C) ruvB* Δ*uvrD recG*

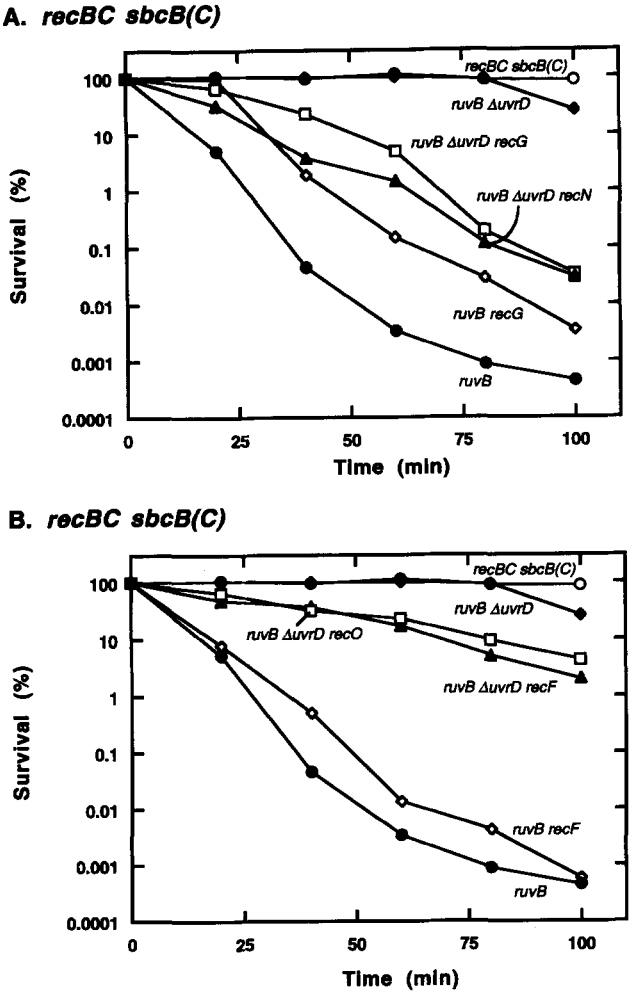

FIGURE 3.—Effect of *recN1502*, *recO1504*, *recF143* and *recG263* mutations on the MMS resistance of the *recBCsbcB(C) ruvB9* Δ*uvrD* cell strain. Stationary cells were exposed to 24 mM MMS for the time periods indicated and then plated on LB agar with the required antibiotics. (A) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, RDK1645 (*ruvB9*); ◆, SWM2054 (*ruvB9* Δ*uvrD*); ◇, SWM2301 (*ruvB9 recG263*); □, SWM3054 (*ruvB9* Δ*uvrD recG263*); ▲, SWM3053 (*ruvB9* Δ*uvrD recN1502*). (B) *recBCsbcB(C)* background: ○, JC7623 [*recBCsbcB(C)*]; ●, RDK1645 (*ruvB9*); ◆, SWM2054 (*ruvB9* Δ*uvrD*); ◇, SWM2300 (*ruvB9 recF143*); □, SWM3052 (*ruvB9* Δ*uvrD recO1504*); ▲, SWM3051 (*ruvB9* Δ*uvrD recF143*). The data presented represents the average of at least four independent experiments.

strain was still higher than that observed for a *recBCsbcB(C) ruvB recG* mutant (Table 3).

Introduction of *recF*, *recO* and *recR* mutations in a *recBCsbcB(C) ruvB* Δ*uvrD* strain had no effect on recombination proficiencies (Table 3 and data not shown). However, the *recF*, *recO* and *recR* mutations in the *ruvB* Δ*uvrD* strain did slightly decrease the repair proficiency of the *recBCsbcB(C) ruvB* Δ*uvrD* mutant, as monitored by repair of MMS-damaged DNA (Figure 3 and data not shown).

Consequences of deleting helicase II in a *recG* mutant: The *recG* gene has recently been shown to encode a DNA helicase (WHITBY *et al.* 1994) involved in the resolution of recombinant products (LLOYD and SHARPLES 1993). This fact, coupled with the knowledge that suppression of the phenotype of a *ruvB* mutation by the *uvrD* deletion was *recG* dependent, prompted us to investigate the effect of a *uvrD* deletion in a *recG* background. We constructed *recG* Δ*uvrD* double mutants in a *recBCsbcB(C)* background and analyzed them for recombination and DNA repair proficiency (Table 4 and Figure 4). Synergistic interactions were detected between *uvrD* and *recG* in both DNA repair and conjugational recombination proficiency assays (Table 4 and Figure 4). The double Δ*uvrD recG* mutant exhibited an increased MMS sensitivity (Figure 4) and an increase in the Hfr-mediated recombination deficiency as compared with either of the single mutant strains (Table 4). This synergistic interaction between helicase II and the RecG protein is not specific for the *recBCsbcB(C)* background but was also observed in the rec^+ sbc^+ background (data not shown).

DISCUSSION

In an effort to understand the role of helicases II and IV in the RecF recombination pathway, we constructed double mutants with either a *helD* (helicase IV) or *uvrD* (helicase II) deletion mutation and a mutation in one of several previously characterized RecF pathway genes (*recF*, *recO*, *recN* and *ruvB*). The results presented above reveal synergistic interactions between helicase II and the RecF, RecO and RecN proteins. These synergistic interactions were, for the most part, evident in both recombinational repair and conjugational recombination. On the other hand, no cooperative interactions were observed between helicase IV and the RecF, RecO and RecN proteins when conjugational recombination proficiency was measured. However, synergistic interactions between helicase IV and the RecF and RecO proteins were detected when recombinational repair was analyzed. It should be noted that the effect with the *recF* mutant was observed only when the repair of MMS-damaged DNA was assayed. Previous studies detected a role for helicase IV in the repair of UV-damaged DNA in Δ*recQ* Δ*helD* double mutants (MENDONCA *et al.* 1995). Therefore, depending on the DNA substrate involved in recombination and the genotype of the cell, there may or may not be a requirement for helicase IV in the repair of damaged DNA. This dependence, or lack thereof, on helicase IV makes it difficult to place *helD* in a specific epistasis group. Synergistic interactions between *helD* and the other RecF pathway genes appear to be dependent on the assay used to monitor recombination or repair proficiency.

The data presented here also reveal the surprising result that a Δ*uvrD* mutation in a *recBCsbcB(C) ruvB* mutant completely suppresses the effect of the *ruvB* mutation. Defects in both the repair of MMS-damaged

TABLE 4

Effect of Δ*uvrD* on *recG* mutants as assayed by conjugational recombination

Strain	Relevant genotype	Relative viability[a]	Relative yield of Thr$^+$ Leu$^+$ transconjugants[a]
JC7623	*recBC sbcB(C)*	1.0 = 4.13×10^8	1.0 = 6.7×10^7
JC7623 Derivatives			
N2730	*recG258*	0.08 ± 0.009	0.002 ± 0.0007
SWM2055	*recG258* Δ*uvrD*	0.23 ± 0.016	0.0001 ± 0.00004
SWM2004	*recG263*	0.19 ± 0.01	0.008 ± 0.0003
SWM2056	*recG263* Δ*uvrD*	0.61 ± 0.012	0.0004 ± 0.00002

Matings were performed in LB media at 37°C for 60 min with donor Hfr JC158 and the appropriate recipient cultures grown to an A_{600} of 0.4 (~2×10^8 cells per ml as determined by viable count) before mixing. Derivative values are means ± SD.

[a] The values for viability and transconjugants given are relative to JC7623 strains mated in parallel (see MATERIALS AND METHODS) and are the means of at least two to six independent sets of experiments. The values of JC7623 the control strain (set equal to 1) are per milliliter of recipient culture (viability) or mating mixture (transconjugants).

DNA and in conjugational recombination are efficiently suppressed. Moreover, suppression of the *ruvB* phenotype by the Δ*uvrD* allele is dependent on the products of the *recG* and *recN* genes and is not dependent on the products of the *recF*, *recO* or *recR* genes. To begin to understand the basis for this effect the biochemical roles of these various proteins must be considered. Genetic and biochemical data suggest an accessory role for the RecF, RecO and RecR proteins in synapsis, perhaps assisting RecA protein to overcome the inhibitory effects of SSB, and allowing RecA to use SSB coated ssDNA as a recombinogenic substrate (UMEZU *et al.* 1993; SANDLER and CLARK 1994). The RecN protein, although not yet purified and analyzed biochemically, has been suggested to be the functional equivalent of RecJ protein, a ssDNA nuclease (LLOYD and BUCKMAN 1991). One explanation for the *recF/O/R* independent suppression of *ruvB* by the Δ*uvrD* mutation presumes a presynaptic role for helicase II, *i.e.*, before the action of the RecF, RecO and RecR proteins. Perhaps helicase II functions in a presynaptic step to generate ssDNA. The coupled activities of a helicase and a nuclease to generate recombinogenic ssDNA ends has been suggested to be the mode of action of RecJ (a 5′ to 3′

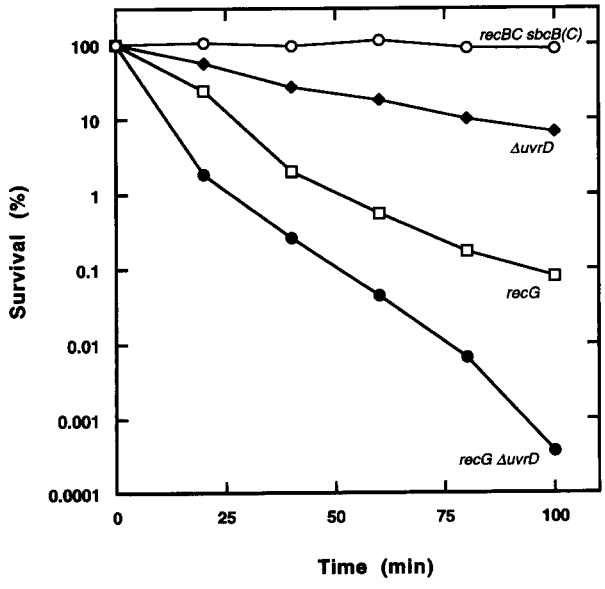

FIGURE 4.—Analysis of *recG263* and Δ*uvrD* single and double mutations on the MMS resistance of a *recBCsbcB(C)* cell strain. Stationary cells were exposed to 24 mM MMS for the time periods indicated and then plated on LB agar with the required antibiotics. ○, JC7623 [*recBCsbcB(C)*]; ◆, SWM2002 (Δ*uvrD*); □, N2730 (*recG258*); ●, SWM2055 (*recG258* Δ*uvrD*). The data represents the average of at least three independent experiments.

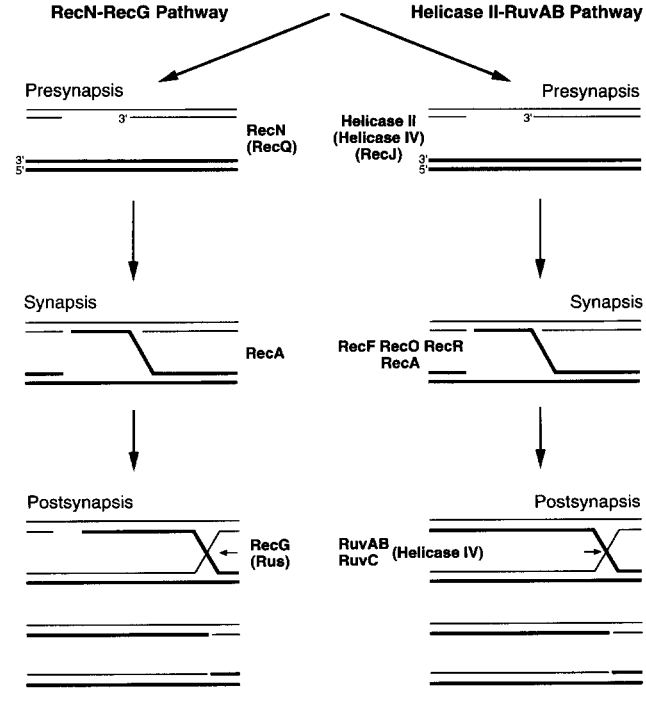

FIGURE 5.—Model of conjugational recombination in the RecF background [*recBCsbcB(C)*] showing the two different pathways of recombination. See text for details.

ssDNA nuclease) and RecQ (a 3′ to 5′ helicase; or alternatively helicase II and helicase IV) (UMEZU *et al.* 1990; MENDONCA *et al.* 1993). If this were the case, then suppression of the *ruvB* phenotype by a Δ*uvrD* mutation could be explained as follows: recombinogenic ssDNA ends generated by helicase II (possibly in combination with a nuclease) can only enter a RecA-RuvAB mediated recombination pathway. Eliminating helicase II would prevent the DNA substrates from entering the helicase II/RuvAB pathway and allow processing of the ssDNA ends through an alternate RecN-RecG mediated recombination pathway. Purified RecG protein is a DNA helicase (WHITBY *et al.* 1994) and has been shown to be capable of catalyzing branch migration (LLOYD and SHARPLES 1993). Thus the RecG protein could compensate for the absence of RuvAB helicase. This would explain the dependence of the suppression on the product of *recG*. Moreover, if the nuclease involved in this alternate pathway were RecN, then the dependence of the suppression on the *recN* gene product would be explained.

This interpretation supports and extends previous studies suggesting the existence of two distinct "RecF" pathways of recombination (CLARK 1991; LLOYD and BUCKMAN 1991). A schematic view of the two RecF pathways is presented in Figure 5. In one pathway, helicase II participates in a presynaptic step, perhaps together with the RecJ nuclease, to generate recombinogenic ssDNA. Helicase II has been shown to interact with the RecJ nuclease, presumably to generate ssDNA, in the methyl-directed mismatch repair pathway (MODRICH 1989). An interaction between helicase II and the RecJ nuclease would extend the functional coupling of these two proteins to a role in recombination. The RecF, RecO and RecR proteins, together with the RecA protein, function in synapsis as previously described (UMEZU *et al.* 1993; SANDLER and CLARK 1994). Finally the recombinant products are processed and resolved by the RuvA, B, C proteins. In the other pathway, depicted on the left, the recombinogenic ssDNA ends are produced by the RecN protein, perhaps in conjunction with the RecQ helicase. This would be consistent with genetic data that places *recQ* and *recN* genes in the same epistasis group, and *recQ* and *recF/O* genes in different epistasis groups (NAKAYAMA *et al.* 1985; LLOYD and BUCKMAN 1991). Again, the RecA protein mediates synapsis. However, in this pathway the recombinants are processed by the RecG helicase (WHITBY *et al.* 1994) and probably the Rus protein. Rus protein, encoded by the *rus* gene, was recently identified as a *recG* dependent suppressor of *ruv* (MANDAL *et al.* 1993). The *rus* mutation probably functions by increasing the expression of an activity, which helps resolve recombination intermediates in conjunction with the RecG protein (MANDAL et al. 1993).

The synergistic interactions observed in Δ*uvrD recG* and the Δ*uvrD recN* double mutants in this study, and those previously reported for the *recF, recO, recR* genes and the *recN* genes (LLOYD and BUCKMAN 1991), support the existence of two separate pathways. Alternatively, helicases II, IV and RecQ could be functioning in three separate recombination pathways. However, genetic analysis has shown a single helicase IV mutant to be recombination proficient and a double helicase II/helicase IV mutant to be recombination deficient (MENDONCA *et al.* 1993). Therefore, it is more likely that the synergistic interactions observed between helicase II and helicase IV, the RecF, the RecO and the RecR proteins are due to partial blocks of the RuvAB-helicase II recombination pathway resulting in the observed decrease in recombination and repair efficiency. Moreover, when we consider recombinational repair, it is apparent that the *recF* and *recO* mutations do decrease repair efficiency to a small extent in a *recBCsbcB(C) ruvB* Δ*uvrD* mutant. This would appear to weaken the argument for two distinct mechanisms of recombination in a *recBCsbcB(C)* background. However, if the data were to be analyzed with respect to the *ruvB* mutant, that is, if we look at the suppression of the repair deficiency of a *recBCsbcB(C) recF* Δ*uvrD* mutant by a *ruvB* mutation, the suppression is almost complete. This also holds true for the suppression of the recombination and repair deficiency of the Δ*uvrD recO* and the Δ*uvrD recR* mutations (data not shown) by the *ruvB* mutation. This then lends further support to the hypothesis of two distinct mechanisms of recombination in a *recBCsbcB(C)* background.

The notion of two pathways may also help begin to explain why *ruv* mutants are deficient in repair in a wild-type background (LLOYD *et al.* 1984). The presence of helicase II could hinder the processing of DNA substrates via the RecN-RecG dependent pathway of recombinational repair. If this were the case, then eliminating helicase II in a *recBC*$^{+}$ *sbc*$^{+}$ *ruv* background would be predicted to make the cells repair proficient. Furthermore, repair should be dependent on RecG helicase and the RecN protein. This has yet to be determined. Clearly the notion of two distinct recombinational DNA repair pathways operating in a wild-type cell opens new possibilities that can be readily tested.

Within the context of distinct RecF pathways, two additional observations can be made. First, elimination of helicase II alone is not sufficient to divert recombination into the alternate pathway, the presence of the *ruvB* mutation is also required. If the Δ*uvrD* mutation alone was sufficient for redirecting recombination into an alternate pathway, then the Δ*uvrD* mutation would also suppress the *recF, recO* or the *recR* mutations. This suggests that some alternate helicase (*e.g.*, helicase IV), with or without the RecJ nuclease, may be able to process the DNA substrates to generate recombinogenic ssDNA ends in the absence of helicase II. Second, the recombination and repair efficiency of the *recBCsbcB(C) ruvB* Δ*uvrD recG* mutant, though lower than that ob-

served for a *recBCsbcB(C) ruvB* Δ*uvrD* mutant, is still higher than the recombination and repair efficiency of the *recBCsbcB(C) ruvBrecG* mutant. This suggests that there must be an alternate protein(s) capable of mediating branch migration of heteroduplex DNA and resolution of recombination intermediates in the *recBCsbcB(C)ruvB* Δ*uvrD recG* mutant. The decreased recombination efficiency seen in a *recBCsbcB(C)ruvB* Δ*helD* mutant suggests that maybe helicase IV, in the absence of helicase II, is capable of carrying out branch migration *in vivo* partially compensating for the absence of RuvAB and RecG. *In vitro* experiments have shown that helicase IV is able to eliminate recombination intermediates formed by the RecA protein (V. M. MENDONCA and S. W. MATSON, unpublished observations). Therefore a partial functional compensation of the RuvAB helicase and RecG helicase by helicase IV is possible.

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LITERATURE CITED

ARTHUR, H. M., and R. G. LLOYD, 1980 Hyper-recombination in *uvrD* mutants of *Escherichia coli* K-12. Mol. Gen. Genet. **180:** 185–191.

CLARK, A. J., and A. D. MARGULIES, 1965 Isolation and characterization of recombination deficient mutants of *E. coli* K12. Proc. Natl. Acad. Sci. USA **53:** 451–459.

CLARK, A. J., 1973 Recombination deficient mutants of *E. coli* and other bacteria. Annu. Rev. Genet. **7:** 67–86.

CLARK, A. J., 1991 *rec* genes and homologous recombination proteins in *Escherichia coli*. Biochimie **73:** 523–532.

COHEN, A., and A. J. CLARK, 1986 Synthesis of linear plasmid multimers in *Escherchia coli*. J. Bacteriol. **167:** 327–335.

HORII, Z.-I., and A. J. CLARK, 1973 Genetic analysis of the RecF pathway to genetic recombination in *Escherchia coli* K-12: isolation and characterization of mutants. J. Mol. Biol. **80:** 327–344.

HOWARD-FLANDERS, P., R. P. BOYCE and L. THERIOT, 1966 Three loci in *Escherichia coli* K-12 that control the excision of pyrimidine dimers and certain other mutagen products from DNA. Genetics **53:** 1119–1136.

KOLODNER, R. D., R. FISHEL and M. HOWARD, 1985 Genetic recombination of bacterial plasmid DNA: effect of RecF pathway mutations on plasmid recombination in *Escherichia coli*. J. Bacteriol. **163:** 1060–1066.

KUSHNER, S. R., J. SHEPHERD, G. EDWARDS and V. F. MAPLES 1978 *uvrD, uvrE* and *recL* represent a single gene, pp. 251–254 in *DNA Repair Mechanisms*, edited by P. C. HANAWALT, E. C. FREIDBERG and C. F. FOX. Academic Press, New York.

LLOYD, R. G., and C. BUCKMAN, 1991 Overlapping functions of *recD, recJ* and *recN* provide evidence of three epistatic groups of genes in *Escherichia coli* recombination and DNA repair. Biochimie **73:** 313–320.

LLOYD, R. G., and G. J. SHARPLES, 1992 Genetic analysis of recombination in prokaryotes. Curr. Opin. Genet. Dev. **2:** 683–690.

LLOYD, R. G., and G. J. SHARPLES, 1993 Dissociation of synthetic holliday junctions by *E. coli* RecG protein. EMBO J. **12:** 17–22.

LLOYD, R. G., F. E. BENSON and C. E. SHURVINGTON, 1984 Effect of *ruv* mutations on recombination and DNA repair in *Escherichia coli* K-12. Mol. Gen. Genet. **194:** 303–309.

LLOYD, R. G., N. P. EVANS and C. BUCKMAN, 1987 Formation of recombinant *lacZ*$^+$ DNA in conjugational crosses with a *recB* mutant of *Escherchia coli* K12 depends on *recF, recJ,* and *recO*. Mol. Gen. Genet. **209:** 135–141.

LUISI-DELUCA, C., S. T. LOVETT and R. D. KOLODNER, 1989 Genetic and physical analysis of plasmid recombination in *recB recC sbcB* and *recB recC sbcA Escherichia coli* K-12 mutants. Genetics **122:** 269–278.

MANDAL, T. N., A. A. MAHDI, G. J. SHARPLES and R. G. LLOYD, 1993 Resolution of Holliday intermediates in recombination and DNA repair: indirect suppression of *ruvA, ruvB,* and *ruvC* mutations. J. Bacteriol. **175:** 4325–4334.

MENDONCA, V. M., K. KAISER-ROGERS and S. W. MATSON, 1993 Double Helicase II (*uvrD*)-Helicase IV (*helD*) deletion mutants are defective in the recombination pathways of *Escherichia coli*. J. Bacteriol. **175:** 4641–4651.

MENDONCA, V. M., H. KLEPIN and S. W. MATSON, 1995 DNA helicases in recombination and repair: construction of Δ*uvrD* Δ*helD* Δ*recQ* mutant deficient in recombination and repair. J. Bacteriol. **177:** 1326–1335.

MILLER, J. H., 1972 *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

NAKAYAMA, H., K. NAKAYAMA, R. NAKAYAMA, N. IRINO, Y. NAKAYAMA *et al.*, 1984 Isolation and genetic characterization of a thymineless death-resistant mutant of *Escherichia coli* K12: identification of a new mutation (*recQ1*) that blocks the RecF recombination pathway. Mol. Gen. Genet. **195:** 474–480.

NAKAYAMA, K., N. IRINO and H. NAKAYAMA, 1985 The *recQ* gene of *Escherichia coli*: molecular cloning and isolation of insertion mutants. Mol. Gen. Genet. **200:** 266–271.

ORREN, D. K., C. P. SELBY, J. E. HEARST and A. SANCAR, 1992 Post-incision steps of nucleotide excision repair in *Escherchia coli*. J. Biol. Chem. **267:** 780–788.

ROSENBERG, S. M., and P. J. HASTINGS, 1991 The split-end model for homologous recombination at double-stranded breaks and at Chi. Biochimie **73:** 365–373.

RYDER, L., M. C. WHITBY and R. G. LLOYD, 1994 Mutation of *recF, recJ, recO, recQ,* or *recR* improves Hfr recombination in resolvase-deficient *ruv recG* strains of *Escherichia coli*. J. Bacteriol. **176:** 1570–1577.

SAMBROOK, J., E. R. FRITSCH and R. MANIATIS, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.

SANCAR, A., and G. B. SANCAR, 1988 DNA repair enzymes. Annu. Rev. Biochem. **57:** 29–67.

SANDLER, S., and A. J. CLARK, 1994 RecOR suppression of *recF* mutant phenotypes in *Escherichia coli* K-12. J. Bacteriol. **176:** 3661–3672.

TSANEVA, I. R., B. MULLER and S. C. WEST, 1993 RuvA and RuvB proteins of *Escherichia coli exhibit* DNA helicase activity *in vitro*. Proc. Natl. Acad. Sci. USA **90:** 1315–1319.

UMEZU, K., K. NAKAYAMA and H. NAKAYAMA, 1990 *Escherichia coli* RecQ protein is a DNA helicase. Proc. Natl. Acad. Sci. USA **878:** 5363–5367.

UMEZU, K., N. CHI and R. D. KOLODNER, 1993 Biochemical interaction of *Escherichia coli* RecF, RecO and RecR proteins with RecA and single-stranded DNA binding protein. Proc. Natl. Acad. Sci. USA **90:** 3875–3879.

WHITBY, M. C., S. D. VINCENT and R. G. LLOYD, 1994 Branch migration of Holliday junctions: identification of RecG protein as a junction specific DNA helicase. EMBO J. **13:** 5220–5228.

WILSON, K., 1989 Preparation of genomic DNA from bacteria, pp. 2.4.1–2.4.5 in *Current Protocols in Molecular Biology*, edited by F. M. AUSUBEL. John Wiley and Sons, Inc., New York.

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