JOURNAL OF BACTERIOLOGY, Aug. 1993, p. 4641–4651
0021-9193/93/154641-11$02.00/0

Vol. 175, No. 15

Double Helicase II (*uvrD*)-Helicase IV (*helD*) Deletion Mutants Are Defective in the Recombination Pathways of *Escherichia coli*

VEERA M. MENDONCA,[1] KATHLEEN KAISER-ROGERS,[2]† AND STEVEN W. MATSON[1,2]*

Department of Biology[1] and Curriculum in Genetics,[2] University of North Carolina, Chapel Hill, North Carolina 27599

Received 16 February 1993/Accepted 30 May 1993

The *Escherichia coli helD* (encoding helicase IV) and *uvrD* (encoding helicase II) genes have been deleted, independently and in combination, from the chromosome and replaced with genes encoding antibiotic resistance. Each deletion was verified by Southern blots, and the location of each deletion was confirmed by P1-mediated transduction. Cell strains containing the single and double deletions were viable, indicating that helicases II and IV are not essential for viability. Cell strains lacking helicase IV (Δ*helD*) exhibited no increase in sensitivity to UV irradiation but were slightly more resistant to methyl methanesulfonate (MMS) than the isogenic wild-type cell strain. As expected, cell strains containing the helicase II deletion (Δ*uvrD*) were sensitive to both UV irradiation and MMS. The introduction of the helicase IV deletion into a Δ*uvrD* background had essentially no effect on the UV and MMS sensitivity of the cell strains analyzed. The double deletions, however, conferred a Rec⁻ mutant phenotype for conjugational and transductional recombination in both *recBC sbcB*(*C*) and *recBC sbcA* backgrounds. The Rec⁻ mutant phenotype was more profound in the *recBC sbcB*(*C*) background than in the *recBC sbcA* background. The recombination-deficient phenotype indicates the direct involvement of helicase II and/or helicase IV in the RecF pathway [*recBC sbcB*(*C*) background] and RecE pathway (*recBC sbcA* background) of recombination. The modest decrease in the recombination frequency observed in single-deletion mutants in the *recBC sbcB*(*C*) background suggests that either helicase is sufficient. In addition, helicase IV has been overexpressed in a tightly regulated system. The data suggest that even modest overexpression of helicase IV is lethal to the cell.

DNA helicases, which catalyze the nucleoside triphosphate hydrolysis-dependent unwinding of duplex DNA, play a crucial role in all aspects of DNA metabolism (for reviews, see references 27 and 28). In *Escherichia coli* alone, there exist at least 10 distinct helicases which have been purified and characterized (27, 28). A combination of genetic and in vitro biochemical analyses have implicated these enzymes in various facets of nucleic acid metabolism. For example, helicase I participates in F factor-mediated conjugative DNA transfer (1). DnaB, PriA, and Rep proteins participate in DNA replication (23, 24, 34). Helicase II, product of the *uvrD* gene, has been shown to have roles in both methyl-directed mismatch repair and excision repair pathways (30, 32, 37). The latter repair pathway also involves the UvrAB complex, which has been demonstrated to have helicase activity (31). Finally, largely on the basis of genetic data, the different recombination pathways in the cell have been shown to utilize the RecBCD enzyme, helicase II, and the RecQ helicase (15, 44, 47). Helicase III (53) and helicase IV (51) have yet to be assigned a specific cellular function.

Helicase IV was identified and purified on the basis of its DNA unwinding activity and was shown to be a 75-kDa enzyme capable of unwinding duplex DNA in an ATP-dependent reaction (51, 52). The polarity of DNA unwinding is in the 3′-to-5′ direction with respect to the bound DNA strand (51). The gene encoding helicase IV, *helD*, was subsequently cloned, sequenced, and mapped to approximately 22 min on the *E. coli* chromosome (52). Since helicase IV was identified solely on the basis of its biochemical activity, there are no genetic data to complement the in vitro studies and no clear indication as to what role helicase IV plays in the cell. It has been noted that helicase IV shares several physical and biochemical properties with helicase II and Rep protein. In addition, the helicase IV protein contains significant amino acid homology with both helicase II and Rep protein (52). In this communication, we report the effect of overexpression of helicase IV and present a partial characterization of its in vivo activity on the basis of genetic studies of single *helD* and double *helD-uvrD* mutants.

MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains, plasmids, and bacteriophages used in this study are listed in Table 1. Genotype designations are those of Bachmann (4). All of the strains involved in recombination and repair assays were derivatives of AB1157 constructed by bacteriophage P1 transduction as described by Miller (29). The identification of Δ*helD*::*cam* and Δ*uvrD*::*tet* mutants among the transductants was accomplished by screening for the appropriate antibiotic resistance, with subsequent analysis of their co-transduction frequencies: *helD* with *pyrD* (38 to 42%) and *uvrD* with *metE* (50%). The helicase II deletions were also checked for their sensitivity to UV irradiation. The deletion strains were ultimately confirmed by Southern analysis. The construction of plasmids pET9d-H4, pT73ΔH4::cam, and pBSΔH2(X) is described below.

Media and methods. Luria-Bertani medium and M56/2 agar and medium were prepared as previously described (49). Medium was supplemented when required with tetracycline (7 μg/ml), chloramphenicol (25 μg/ml), and appropriate amino acids (40 μg/ml). All other supplements and antibiotics were used at the concentrations recommended by Sam-

* Corresponding author.
† Present address: Department of Pediatrics, University of North Carolina, Chapel Hill, NC 27599.

TABLE 1. Bacterial strains, plasmids, and bacteriophages

Strain designation	Significant feature(s)	Source, reference, or derivation
Cell strains		
C600	*supE44 hsdR thi-1 thr-1 leuB6 lacY1 tonA21* r_{k-} m_{k+}	2
HB101	*supE44 hsd20* r_{B-} m_{B-} *recA13 ara14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*	7
RW592	HfrH Δ(*gal-bio*) *thi* Str^s su^-	P. Bassford
RW592ΔH2	Δ*uvrD*::*tet*	This study
GE1721[a]	*pyrD*::*cam*	G. Weinstock
GE1752[b]	*metE*::*cam*	G. Weinstock
GE1721ΔH4	$pyrD^+$ Δ*helD*::*cam*	P1.JC7623ΔH4 × GE1721 to $PyrD^+$, Cam^r
GE1752ΔH2	$metE^+$ Δ*uvrD*::*tet*	P1.RWΔH2 × GE1752 to $MetE^+$, Tet^r
GEΔH2ΔH4	Δ*uvrD*::*tet* Δ*helD*::*cam*	P1.GEΔH4 × GEΔH2 to Cam^r, Tet^r
HMS174	*recA* r_{k12-} m_{k12-} Rif^r	Novagen
HMS174(DE3)	λDE3	Novagen
JC158	HfrP01 *lacI22* λ^- *serA6 spoT1 thi-1*	10
AB1157[c]	F^- *thr-1 leuB6 thi-1 lacY1 galK2 ara14 xyl5 mtl-1 proA2 his4 argE3 rpsL3* (Sm^r) *tsx-33 supE44 kdgK51*	16
AB1157 derivatives[c]		
AB2463	*recA13*	17
AB1157ΔH4	Δ*helD*::*cam*	P1.GE1721ΔH4 × AB1157 to Cam^r
AB1157ΔH2	Δ*uvrD*::*tet*	P1.GE1752ΔH2 × AB1157 to Tet^r
AB1157ΔH2ΔH4	Δ*helD*::*cam* Δ*uvrD*::*tet*	P1.GE1721ΔH4 × ABΔH2 to Cam^r, Tet^r
JC7623	*recB21 recC22 sbcB15 sbcC201*	11
JC7623ΔH4	Δ*helD*::*cam*	This study
JC7623ΔH2	Δ*uvrD*::*tet*	P1.GE1752ΔH2 × JC7623 to Tet^r
JC7623ΔH2ΔH4	Δ*helD*::*cam* Δ*uvrD*::*tet*	P1.GE1752ΔH4 × JC7623ΔH2 to Cam^r, Tet^r
JC8679	*recB21 recC22 sbcA23*	14
JC8679ΔH4	Δ*helD*::*cam*	P1.GE1721ΔH2 × JC8679 to Cam^r
JC8679ΔH2	Δ*uvrD*::*tet*	P1.GE1752ΔH2 × JC8679 to Tet^r
JC8679ΔH2ΔH4	Δ*helD*::*cam* Δ*uvrD*::*tet*	P1.GE1752ΔH4 × JC8679ΔH2 Cam^r, Tet^r
Plasmids		
pET9d	From pBR322, Kan^r T7 expression vector	Novagen (41)
pET9d-H4	Kan^r, *helD* contained within a 2.2-kb *Nco*I-*Bam*HI fragment	This study
pLysS	From pACYC184, Cam^r encoding T7 lysozyme in the opposite orientation of the *tet* promoter	Novagen (41)
pLysE	As pLysS, but T7 lysozyme encoded in the same orientation as the *tet* promoter	Novagen (41)
pDJ17	Amp^r ColE1 origin *lacZα*::*cam*	P. Berget
pT73	Amp^r ColE1 origin	42
pT73H4	Amp^r *helD* contained within a 4.0-kb *Pst*I fragment	This study
pT73ΔH4	As pT73H4, but lacks a 1.3-kb *Sma*I fragment containing the *helD* gene sequence	This study
pT73ΔH4::cam	As pT73ΔH4, but the deleted *Sma*I fragment has been replaced with a 1.4-kb Cam^r gene	This study
pBR322	Amp^r Tet^r	6
pBluescript M13	Amp^r	38
pBSRV.5	As pBluescript, but with *uvrD* contained within a 5-kb *Eco*RV fragment	This study
pGT26	Amp^r Tet^r *uvrD* contained within a 2.9-kb *Pvu*II fragment	43
pGT26Δtet	As pGT26, but Tet^s	This study
pGT26ΔtetΔH2	Amp^r Tet^s *uvrD* flanking sequence contained within a 0.46-kb *Pvu*II fragment	This study
pGT26ΔH2::tet	Amp^r Δ*uvrD*::*tet* contained within a 1.2-kb *Pvu*II fragment	This study
pTA108	Amp^r pSC101 origin	45
pTAH2	As pTA108, but *uvrD* contained within a 2.9-kb *Pvu*II fragment	This study
Bacteriophages		
λCh80de19	φ80 λ^s	P. Bassford
λgt1-λB	Δ*attP cI857*	C. Joyce
λgt1-ΔH2::tet	As λgt1-λB but Δ*uvrD*::*tet* contained on a 4-kb *Xba*I fragment	This study
λvir	$v_1v_2v_3$	P. Bassford
λ12G1(553)	λ2001, with *uvrD* encoded on a large chromosomal *Eco*RI fragment	20

[a] Wild type, except for the *pyrD* marker.
[b] Wild type, except for the *metE* marker.
[c] All AB1157 derivatives are isogenic with AB1157, except for the indicated markers.

brook et al. (33). UV survival and methyl methanesulfonate (MMS) survival assays were performed as previously described (35). Conjugation experiments for determining recombination proficiency were performed as described by Willetts et al. (49). Briefly, matings were performed for 1 h at 37°C with a 10:1 recipient/donor ratio with cells that had been grown to an optical density at 600 nm (OD_{600}) of 0.4 (approximately 2×10^8 cells per ml). Viability of Luria-Bertani broth cultures was determined by comparing viable cells (determined by diluting and plating on duplicate Luria-Bertani and M56/2 plates) to the total number of cells (determined by OD_{600} where an OD_{600} of 0.4 is approximately 2×10^8 cells per ml). Matings were interrupted by vigorous vortexing followed by selection on M56/2 agar medium with the appropriate supplements. Transconjugant selection was for leucine and threonine prototrophy; counterselection was for serine prototrophy and antibiotic resistance. The yield of transconjugants obtained for each strain is expressed as a ratio relative to AB1157. P1 transductions to Leu^+ were performed as described by Miller (29). The P1 transducing lysate was propagated on JC158. The relative transduction frequency is the number of leu^+ transductants obtained for the strain in question relative to the number obtained for a JC7623 strain transduced in parallel.

DNA techniques. All 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. (33), followed by banding in CsCl-ethidium bromide gradients. *E. coli* chromosomal DNA and lambda DNA were prepared as described previously (12, 50). DNA restriction fragment probes were radioactively labelled with the Random Primed DNA Labelling Kit from US Biochemicals and [α-^{32}P]dCTP according to the manufacturer's specifications. Southern blotting was performed as described by Sambrook et al. (33) with GeneScreen nylon membranes.

Chemicals and enzymes. Restriction endonucleases were purchased from New England Biolabs, Inc., and were used as specified by the manufacturer. Isopropyl-β-D-thiogalactopyranoside (IPTG) was purchased from US Biochemicals. MMS was purchased from Sigma. All other chemicals were of reagent or ultrapure grade.

Construction of pET9d-H4. A 4-kb *Pst*I DNA fragment containing the *helD* gene (52) and flanking chromosomal sequence was subcloned into the polylinker region of M13mp18 at the *Pst*I site. Site-directed mutagenesis (22) with a synthetic 20-mer (5'-TCTACAGAGCCATGGAAC TG-3') generated a new *Nco*I site 2 bases upstream of the ATG initiation codon of *helD*. This *Nco*I site and the *Bam*HI site 220 bases downstream of the termination codon of *helD* were cleaved to yield a 2.27-kb DNA fragment containing the *helD* gene. This DNA fragment was inserted into pET9d (Novagen) cleaved with *Nco*I and *Bam*HI to yield pET9d-H4 (Fig. 1). The construction was verified by restriction mapping and partial DNA sequencing.

Construction of pT73ΔH4::cam. pT73ΔH4::cam was constructed as shown in Fig. 2. pT73H4 (6.3 kb) consists of the pT73 vector (kindly provided by Stan Tabor, Harvard Medical School) (42) in which a 4-kb *Pst*I DNA fragment containing the *helD* gene (52) and flanking chromosomal DNA has been cloned. pT73H4 was digested to completion with *Sma*I to remove 1,347 nucleotides of *helD* gene sequence. The resulting ~5.0-kb DNA fragment was isolated, *Xho*I linkers were added, and the fragment was exhaustively digested with *Xho*I. Intramolecular ligation of the ends of this fragment resulted in the formation of pT73ΔH4. A 1.4-kb *Bam*HI fragment containing the chloramphenicol resistance

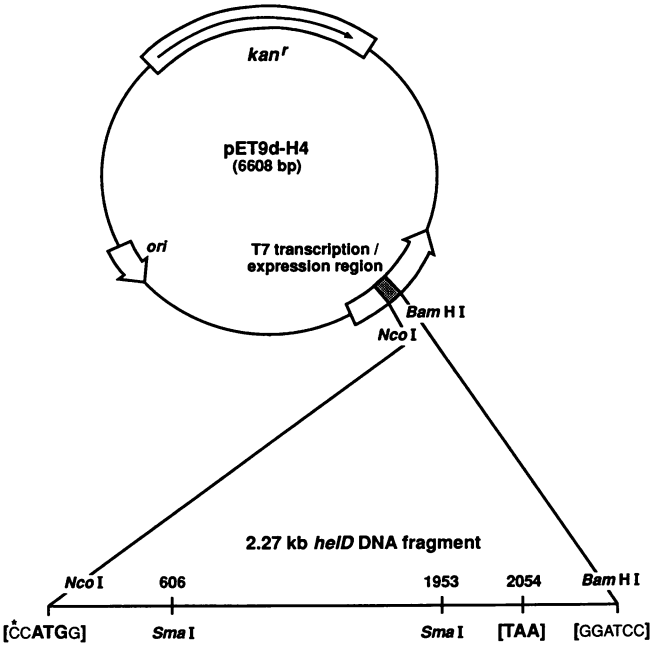

FIG. 1. The helicase IV overexpression plasmid. See text for details on construction of the plasmid. The star denotes the nucleotide changed by site-directed mutagenesis to create an *Nco*I site. TAA denotes the *helD* termination codon.

(Cam^r) gene was isolated from pDJ17 on an agarose gel and purified with GeneClean (Bio 101 Inc.). The ends were filled in with *E. coli* DNA polymerase I (Klenow fragment) and deoxynucleoside triphosphates (dNTPs) and *Xho*I linkers were added. After digestion with *Xho*I, this fragment was ligated to pT73ΔH4 that had been linearized with *Xho*I. The resulting plasmid, pT73ΔH4::cam, contains a Cam^r gene surrounded by 606 nucleotides of 5' *helD* sequence and 101 nucleotides of the 3' *helD* sequence. This plasmid also contains 1.6 kb of upstream and 1.1 kb of downstream chromosomal flanking sequence. The *Xba*I and *Hin*dIII restriction sites present in the polylinker of pT73ΔH4::cam were subsequently used to produce the DNA fragment utilized to effect gene replacement in JC7623 (see below).

Construction of pBSΔH2(X). pBSΔH2(X) was constructed in vitro as shown in Fig. 3. The *uvrD* gene was deleted from pGT26Δtet by cleavage with *Bst*EII followed by digestion with *Bal* 31 nuclease. The resulting linear molecule was then digested with *Bgl*II to remove the upstream regulatory sequence from the *uvrD* gene. The ends of the linear molecule were filled in with *E. coli* DNA polymerase I (Klenow fragment) and dNTPs, and *Xho*I linkers were added. The Tet^r gene used to replace the *uvrD* gene was isolated as a 1.4-kb *Eco*RI-*Ava*I DNA fragment from pBR322, to which *Xho*I linkers were added. This fragment was then ligated to the vector fragment, lacking the *uvrD* gene, to yield pGT26ΔH2::tet. The resulting plasmid was isolated and digested to completion with *Nde*I and *Bss*HII. The *Bss*HII-*Nde*I fragment, containing the Tet^r gene, a small portion of the 3' end of the *uvrD* gene, and short regions of chromosomal DNA flanking the *uvrD* gene (Δ*uvrD*::*tet*), was then isolated. This DNA fragment was used to directly replace the *Bss*HII-*Nde*I fragment containing the wild-type *uvrD* gene in pBSRV.5. pBSRV.5 consists of a modified Bluescript M13 vector, into which a 5-kb DNA fragment

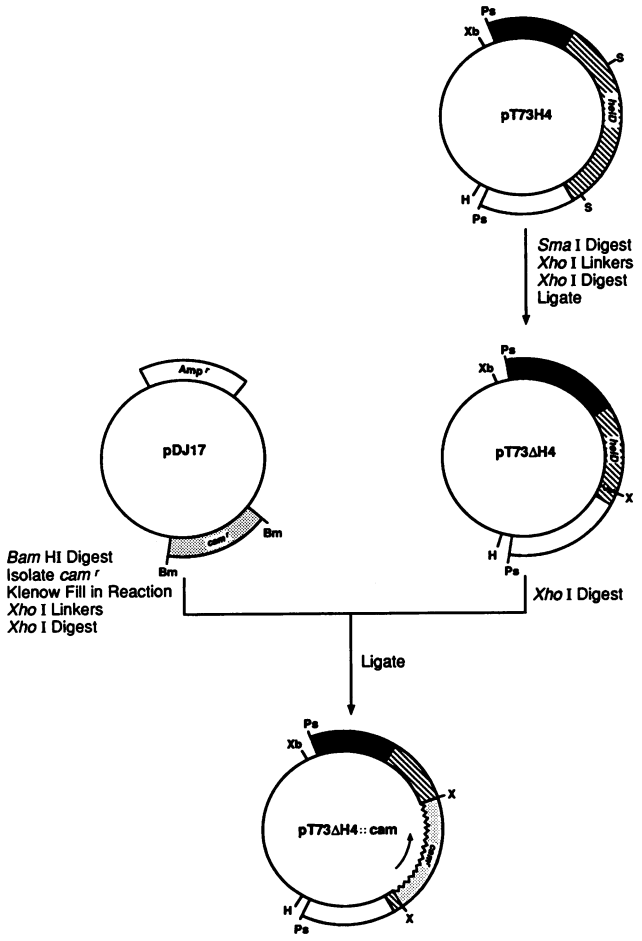

FIG. 2. Construction of pT73ΔH4::cam. pT73H4 was digested with *Sma*I to remove 1,347 nucleotides of internal *helD* sequence. *Xho*I linkers were ligated to each *Sma*I end, and the resulting vector fragment was religated to yield pT73ΔH4. To construct pT73ΔH4::cam, a *Bam*HI fragment containing the Cam^r gene was isolated from pDJ17 and cloned into the *Xho*I site within pT73ΔH4. Hatched areas, *helD* gene sequence; solid areas, 5′ flanking DNA sequence; open areas, 3′ flanking DNA; shaded areas, Cam^r gene. Restriction endonuclease sites: Bm, *Bam*HI; H, *Hin*dIII; Ps, *Pst*I; S, *Sma*I; X, *Xho*I; Xb, *Xba*I.

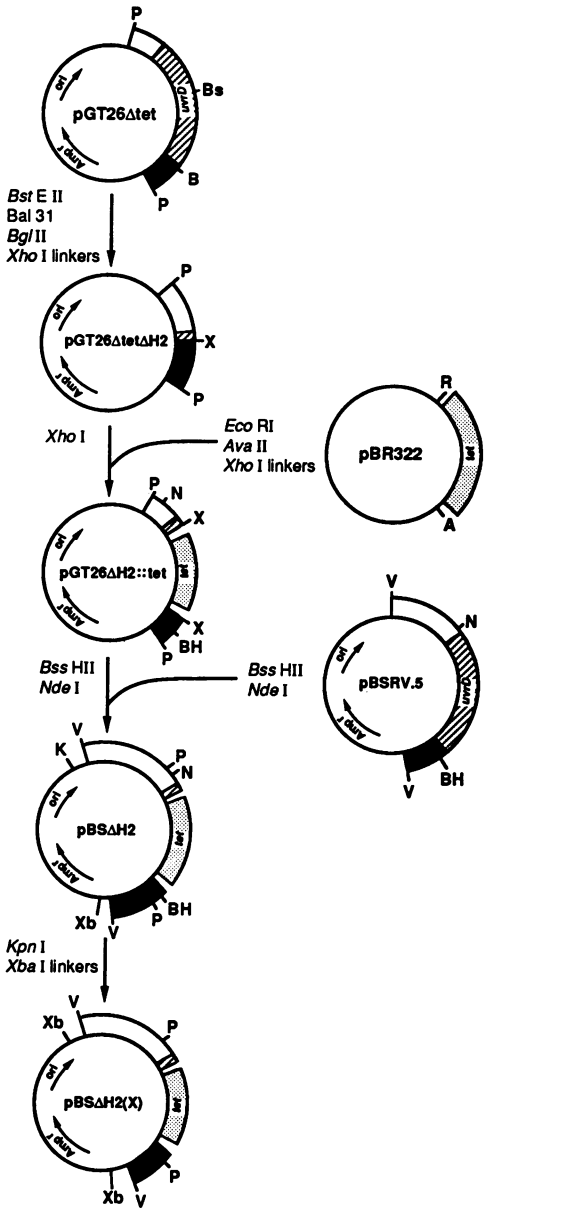

FIG. 3. Construction of pBSΔH2(X). Plasmid pGT26Δtet contains a 2.9-kb *Pvu*II fragment encoding the *E. coli uvrD* gene (hatched areas) as well as the upstream (solid areas) and downstream (open areas) flanking sequences. The upstream regulatory region, as well as the entire *uvrD* gene, with the exception of approximately 30 nucleotides at the 3′ end, was removed from this plasmid as described in Materials and Methods. The *uvrD* gene was then replaced with a 1.4-kb *Eco*RI-*Ava*I fragment containing the Tet^r gene from pBR322 (shaded areas) to yield pGT26ΔH2::tet. The *Nde*I-*Bss*HII fragment was removed from pGT26ΔH2::tet and used to replace the *Nde*I-*Bss*HII fragment in pBSRV.5. The resulting plasmid, pBSΔH2, was subsequently modified by replacing the *Kpn*I site within its polylinker with an *Xba*I linker to permit removal of an *Xba*I cassette containing the *uvrD*::*tet* gene. Restriction endonuclease cleavage sites: A, *Ava*I; B, *Bgl*II; BH, *Bss*HII; Bs, *Bst*EII; R, *Eco*RI; V, *Eco*RV; K, *Kpn*I; N, *Nde*I; P, *Pvu*II; Xb, *Xba*I; X, *Xho*I.

containing the *uvrD* gene as well as extensive regions of upstream and downstream flanking sequence has been cloned. The resulting plasmid, pBSΔH2, contains the Tet^r gene and approximately 30 nucleotides of the 3′ end of the *uvrD* gene, surrounded by chromosomal DNA sequence flanking the *uvrD* gene. In addition, an *Xba*I linker was used to replace the *Kpn*I site in the polylinker region of pBSΔH2 to yield pBSΔH2(X). This *Xba*I site, in conjunction with the *Xba*I site in the polylinker, provided a means by which the Δ*uvrD*::*tet* gene sequence could be removed as a cassette from pBSΔH2(X) for additional manipulation.

In vitro construction of λgt-ΔH2::tet. A lambda phage containing Δ*uvrD*::*tet* was constructed as follows. The Δ*uvrD*::*tet* cassette was removed from pBSΔH2(X) as a 4.0-kb *Xba*I fragment, isolated by agarose gel electrophoresis, and purified with GeneClean (Bio 101 Inc.). The Δ*uvrD*::*tet* cassette was ligated into the *Xba*I site of λgt1-λB. The resulting DNA was packaged with Packagene (Promega) and transfected into *E. coli* RW592. Plaque hybridization (11) was used to identify those plaques containing the Δ*uvrD*::*tet* cassette. The resulting lambda phage is referred to as λgt1-ΔH2::tet.

TABLE 2. Transformation efficiencies of pET9d and pET9d-H4[a]

Plasmid	Bacterial strain	No. of transformants/ μg of DNA (10^4 CFU)
pET9d	HMS174	1.930 ± 0.83
pET9d-H4	HMS174	1.530 ± 0.80
pET9d	HMS174(DE3)	0.480 ± 0.28
pET9d-H4	HMS174(DE3)	0.000 ± 0.00
pET9d	HMS174(DE3)/pLysS	2.150 ± 0.64
pET9d-H4	HMS174(DE3)/pLysS	0.007 ± 0.00
pET9d	HMS174(DE3)/pLysE	4.300 ± 0.85
pET9d-H4	HMS174(DE3)/pLysE	3.650 ± 0.21

[a] Cells were transformed (33) with 70 to 90 ng of the indicated plasmid DNA and plated on Luria-Bertani medium containing the appropriate antibiotics. CFU were counted after an overnight incubation at 37°C. The mean transformation efficiencies were obtained from three independent experiments.

RESULTS

Construction of a plasmid that directs overexpression of helicase IV. Previous studies suggested that helicase IV was poorly expressed and present at low concentrations in the cell (52a). Analysis of the sequence of the *helD* gene revealed a high percentage of rare and infrequently used codons and a poor Shine-Dalgarno sequence, which together may account for the low level of gene expression (52). Our strategy for overexpressing the *helD* gene product was to provide the strong, regulatable T7 promoter and a more optimal ribosome binding site. The resulting plasmid, pET9d-H4, was constructed as described in Materials and Methods and is shown in Fig. 1.

To express the *helD* gene, pET9d-H4 was transformed into HMS174(DE3), a strain containing the T7 RNA polymerase gene controlled by the *lac* promoter (present in the λDE3 lysogen). The transformation data shown in Table 2 suggest that the additional helicase IV produced in uninduced cells [HMS174(DE3)], due to basal levels of T7 RNA polymerase, was sufficient to cause cell death. Inviability was specifically due to plasmid-directed expression of helicase IV, since transformations performed with the vector alone (pET9d) produced viable, plasmid-containing colonies. In addition, both pET9d and pET9d-H4 could be used to transform a cell strain lacking the T7 RNA polymerase gene (HMS174) with similar efficiencies. The level of uninduced T7 RNA polymerase was further decreased by introducing T7 lysozyme (encoded by the pLysS or the pLysE plasmid) to inhibit T7 RNA polymerase (40). The high levels of T7 lysozyme produced by the pLysE plasmid were required to stably maintain the pET9d-H4 construction in the cell. This suggests that even modest overexpression of helicase IV is lethal to the cell. This interpretation was further supported by an examination of several transformants obtained in the presence of pLysS. In each case, moderately to severely truncated forms of helicase IV were produced, suggesting that the cells could not tolerate overexpression of the full-length polypeptide.

IPTG induction of log-phase cells containing pET9d-H4 and pLysE resulted in increased expression of a polypeptide with an M_r of 75,000, the size expected for helicase IV (51). The protein was purified by the previously described protocol (51) with minor modifications and was shown to catalyze both DNA-dependent ATP hydrolysis and DNA unwinding (data not shown). On the basis of both the physical and biochemical criteria, we conclude that the overproduced protein is helicase IV.

Construction of *helD* and *uvrD* deletion mutants. Helicase II and helicase IV share several physical and biochemical properties (52). They also demonstrate significant amino acid sequence homology (52). Helicase II is known to function in methyl-directed mismatch repair and UvrABC excision repair (30, 37). In addition, Horii and Clark (15) detected the involvement of helicase II in the RecF pathway of recombination, and mutations that suppress a *recJ* mutation have been mapped in the *helD* gene (25a). In an attempt to assess the cellular role of helicase IV, and to determine whether one helicase can compensate for the absence of another, single- and double-deletion mutants of both *helD* and *uvrD* were analyzed for UV sensitivity, MMS sensitivity, and recombination proficiency.

A *helD* deletion strain was constructed by the direct gene replacement technique described by Jasin and Schimmel (18). *E. coli* JC7623 was transformed with a linear 4.0-kb *Xba*I-*Hin*dIII DNA fragment isolated from pT73ΔH4::cam (Fig. 2). Within this linear DNA fragment, a significant portion of the *helD* gene has been replaced with a Cam^r gene. The Cam^r gene is surrounded by the remaining *helD* gene sequence (0.61 kb 5′, 0.10 kb 3′) and flanking chromosomal DNA (1.6 kb 5′, 1.1 kb 3′). A double recombination event, involving homologous DNA sequences present within the transformed DNA fragment containing the Δ*helD*::*cam* gene and the chromosomal *helD* gene, results in direct gene replacement. Cells that had acquired the *helD* deletion and the associated chloramphenicol resistance were readily obtained, suggesting that deletion of the *helD* gene is not lethal to the cell.

The *pyrD* locus (21 min) and *helD* locus (22 min) lie within approximately 1 min of each other on the *E. coli* chromosome and should cotransduce with measurable frequency. With P1 transduction, the Δ*helD*::*cam* locus was moved from JC7623ΔH4 to GE1721, a *helD*$^+$ cell strain that carries a *pyrD*::*cam* marker. The deletion was then moved two additional times within GE1721 to ensure that GEΔH4 was isogenic both with the parental cell strain and the helicase II deletion strains that were constructed (see below). Since both donor [JC7623ΔH4(Δ*helD*::*cam*)] and recipient [GE1721 (*pyrD*::*cam*)] cells were Cam^r, PyrD$^+$ transductants were isolated first and then tested for Cam^r. This selection strategy ensured that each Cam^r transductant contained both the wild-type *pyrD* locus and the Δ*helD*::*cam* locus. A 38 to 42% cotransduction frequency was observed between the *pyrD* and the *helD* loci when PyrD$^+$ transductants were screened for the Δ*helD*::*cam* locus by plating onto minimal media containing chloramphenicol. A similar cotransduction frequency (36 to 53%) was observed between *pyrD* and *helD* when the Δ*helD*::*cam* was moved within GE1721. This series of P1 transductions verified that the *helD* deletion within JC7623ΔH4 and GEΔH4 was correctly positioned at approximately 22 min on the *E. coli* chromosome. It also established that the JC7623 background was not required for viability of a cell strain lacking helicase IV. Physical confirmation of the helicase IV deletion was obtained by Southern blot analysis (Fig. 4). On probing *Eco*RI-digested chromosomal DNA with a *helD* probe, the expected 7.5- and 6.3-kb fragments were observed for the parental DNA (Fig. 4B, lane 1). The Cam^r gene, used to replace the *helD* gene, introduced an additional *Eco*RI site at this locus. Southern analysis of the deletion strain yielded a 5.6- and a 0.7-kb fragment instead of the 6.3-kb parental DNA fragment (Fig. 4B, lanes 3 and 4).

The gene replacement strategy employed to delete the *uvrD* gene utilized a specialized lambda phage referred to as λgt1-λB (19). This bacteriophage carries a temperature-

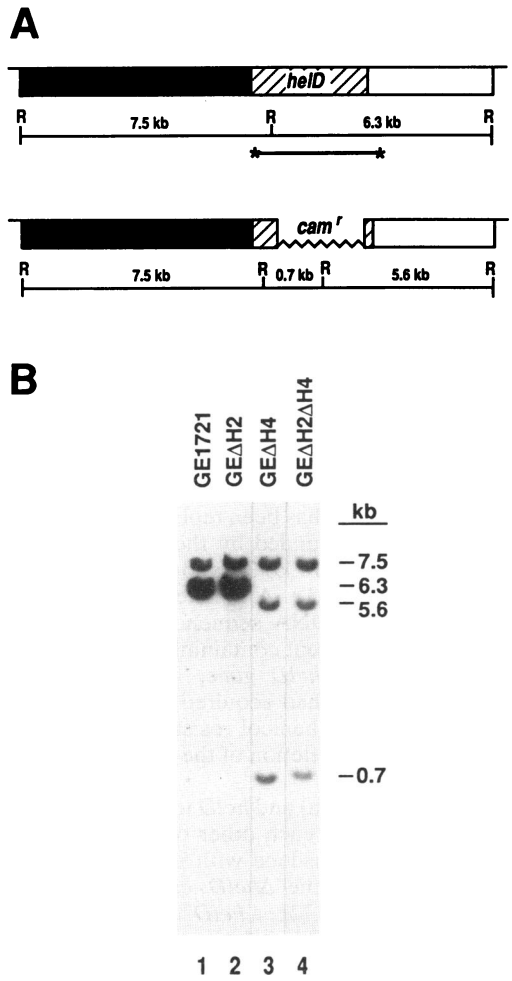

FIG. 4. Southern blot analysis of Δ*helD* strains. (A) A partial physical map of the *E. coli* chromosome is shown before and after deletion of the *helD* gene. The restriction fragments expected after *Eco*RI (R) digestion of the wild-type and mutant *helD* loci are shown. Hatched boxes, *helD* gene; wavy line, Cam^r gene; solid boxes, 5′ flanking chromosomal DNA sequence; open boxes, 3′ flanking chromosomal DNA sequence. (B) Southern blot of chromosomal DNA digested with *Eco*RI and probed with a 2.0-kb *Nco*I-*Bam*HI [^{32}P]DNA fragment containing the *helD* gene and downstream flanking sequence. Lanes: 1, GE1721 (*uvrD*$^+$ *helD*$^+$); 2, GEΔH2 (Δ*uvrD*::*tet*); 3, GEΔH4 (Δ*helD*::*cam*); 4, GEΔH2ΔH4 (Δ*uvrD*::*tet* Δ*helD*::*cam*).

sensitive *c*I repressor gene and is *attP* deficient. For this reason, lysogen formation at 30°C is no longer targeted to the *attB* site on the *E. coli* chromosome. Both the presence of a temperature-sensitive *c*I repressor and the lack of an *attP* site in λgt1-λB were exploited to construct a *uvrD* deletion. A cassette containing a Tet^r gene, approximately 30 nucleotides of the 3′ end of the *uvrD* gene and chromosomal DNA, which normally flanks the *uvrD* gene, was constructed as described in Materials and Methods and is shown in Fig. 3. This cassette was isolated as a 4-kb *Xba*I DNA fragment from pBSΔH2(X) and was ligated into λgt1-λB to produce a lambda phage called λgt1-ΔH2::tet. In the absence of an *attP* site, the chromosomal DNA sequence flanking the *uvrD* gene, present in both the bacterial chromosome and the phage, provides homologous sites which direct phage integration to the *uvrD* locus on the *E. coli* chromosome. Homologous recombination involving either the upstream *uvrD* flanking region (approximately 670 bp) or the downstream flanking region (approximately 1,540 bp) resulted in the formation of the lysogens diagrammed in Fig. 5A. These lysogens were initially identified as Tet^r colonies that demonstrated both lambda immunity in the presence of λvir and λCh80de19, as well as inability to grow at 42°C (the nonpermissive temperature).

Spontaneous excision of the lambda phage from the chromosome, which occurs at a frequency of approximately 10^{-4}, results in the formation of a cell containing either the original wild-type gene or the mutant Δ*uvrD*::*tet* (Fig. 5A). Spontaneously cured cells were selected on the basis of their ability to grow at 42°C in the absence of antibiotic selection. (Note that direct selection for Tet^r, cured cells was unsuccessful. Only lysogens that no longer demonstrated a temperature-sensitive phenotype, presumably because of reversion of the temperature-sensitive *c*I repressor gene, were recovered after direct selection for Tet^r cells.) Each colony was then tested for Tet^r as well as for the loss of lambda immunity. To confirm the presence of the Δ*uvrD*::*tet* allele, DNA from several isolates was analyzed by Southern blot (Fig. 5C and D). *Eco*RV- or *Pvu*II-digested chromosomal DNA was probed with either a *uvrD* or a Tet^r probe. The parental DNA, as expected, showed a 5.0-kb *Eco*RV and a 2.9-kb *Pvu*II band when probed with the *uvrD* probe (Fig. 5C, lanes 1 and 4), and no signal when probed with the Tet^r probe (Fig. 5D, lanes 1 and 4). The Tet^r gene, used to replace the *uvrD* gene, contains an internal *Eco*RV site. A Southern blot of the deletion strains demonstrated the presence of a 3.3- and a 0.7-kb fragment in place of the 5.0-kb *Eco*RV fragment (Fig. 5C, lane 2). The lysogen, which contained both the wild-type and mutant *uvrD* alleles, demonstrated Southern blot patterns similar to those of both the deletion and the parental strains (Fig. 5C, lanes 3 and 6).

Spontaneously cured cells carrying the *uvrD* deletion were readily obtained, suggesting that the *uvrD* gene product is not essential. Alternatively, the loss of helicase II could be compensated for by another gene encoding a protein with a similar function. P1 transductions were used to move the Δ*uvrD*::*tet* locus from the RWΔH2 strain into GE1752, a wild-type strain carrying a *metE*::*cam* marker. The *uvrD*::*tet* locus was moved two additional times within GE1752 to ensure that GEΔH2 was isogenic with the parental cell strain and the helicase IV deletion. Δ*uvrD*::*tet* deletions were isolated by selecting for MetE$^+$ transductants and then screening for Tet^r. Southern analysis performed with the GEΔH2 strains (data not shown) confirmed the presence of the *uvrD* deletion on the *E. coli* bacterial chromosome. Helicase II was also shown to be absent from the deletion strains RWΔH2 and GEΔH2 by Western blots with affinity-purified anti-helicase II antibodies (data not shown). These data confirm similar findings (48) that the *uvrD* gene product is not required for cell viability or that its loss can be compensated for by a functionally similar protein(s).

Neither the *helD* gene nor the *uvrD* gene appears to be essential for cell viability. To test the notion that one helicase may compensate for the absence of another, a double-deletion mutant containing Δ*helD*::*cam* and Δ*uvrD*::*tet* was constructed by P1 transduction. GEΔH2ΔH4 was constructed with GEΔH4 (Δ*helD*::*cam*) as the donor and GEΔH2 (Δ*uvrD*::*tet*) as the recipient strains. Cam^r transductants were readily obtained, and 100% of those tested were also Tet^r, suggesting that there was no selection against formation of the Δ*uvrD*-Δ*helD* double-deletion mutant. The

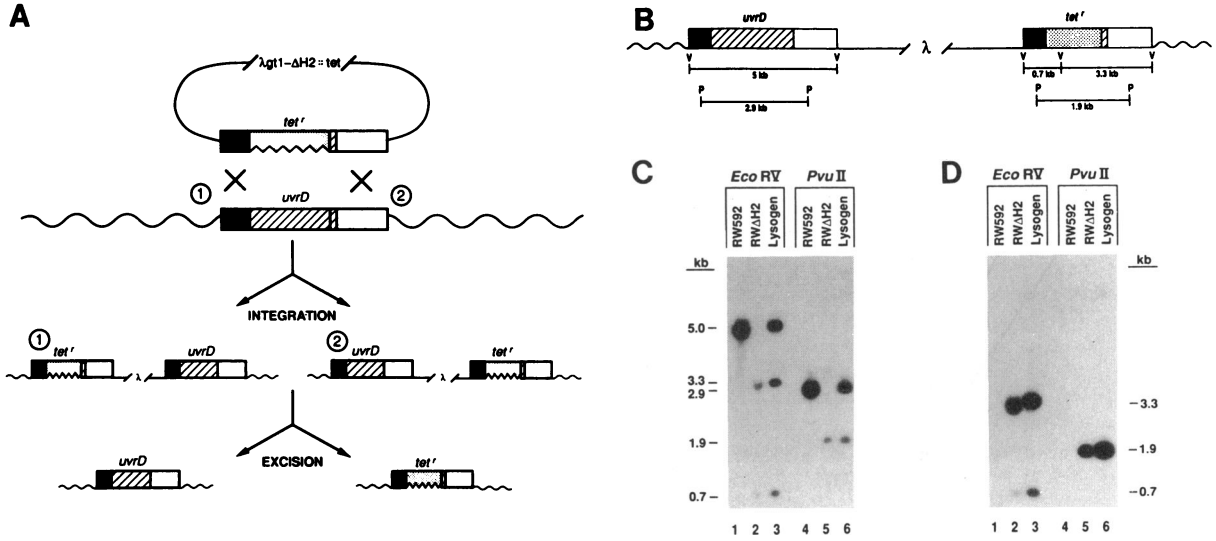

FIG. 5. Deletion of the *uvrD* gene from the *E. coli* chromosome. (A) λgt1-Δ*uvrD*::*tet* phage integration and excision were used to delete the *uvrD* gene from the chromosome of *E. coli* RW592 and replace it with the Δ*uvrD*::*tet* allele. Homologous recombination within the upstream (solid boxes) or the downstream (open boxes) flanking regions of the *uvrD* gene results in the formation of the lysogens diagrammed on the left and right, respectively. Spontaneous curing, with excision of the wild-type gene, produces a cell strain containing the Δ*uvrD*::*tet* gene (*tet*^{r}). (B) A physical map of the λgt1-Δ*uvrD*::*tet* lysogen. Hatched boxes, *uvrD* gene; solid boxes, chromosomal DNA sequence 5′ to *uvrD*; open boxes, chromosomal DNA sequence 3′ to *uvrD*; shaded box, the Tet^r gene. The restriction fragments expected for both *Eco*RV and *Pvu*II digestion of the wild-type and the mutant loci are shown. Restriction endonuclease cleavage sites: P, *Pvu*II; V, *Eco*RV. (C) Chromosomal DNA from RW592, the parental cell strain, RWΔH2, the *uvrD* deletion cell strain, and a lysogen containing both the wild-type and mutant *uvrD* genes was restricted with either *Eco*RV or *Pvu*II. Southern blots of chromosomal DNA from RW592 (lanes 1 and 4), RWΔH2 (lanes 2 and 5), and a λgt1-Δ*uvrD*::*tet* lysogen (lanes 3 and 6) were probed with a 2.9-kb *Pvu*II DNA fragment containing both the *uvrD* gene and flanking DNA sequence. DNA fragment sizes are indicated on the left. (D) Southern blot of chromosomal DNA from RW592 (lanes 1 and 4), RWΔH2 (lanes 2 and 5), and a λgt1-Δ*uvrD*::*tet* lysogen (lanes 3 and 6) probed with a 1.4-kb *Eco*RV-*Ava*I fragment containing only the Tet^r gene sequence. DNA fragment sizes are indicated on the right.

double deletions were confirmed by Southern analysis (data not shown) and by obtaining cotransduction frequencies for *helD* and *uvrD* with *pyrD* and *metE*, respectively. The ability to make these deletions suggests that the *helD* and *uvrD* gene products are not crucial for cell viability or that their loss can be compensated for by a functionally similar protein(s).

UV and MMS resistance phenotypes exhibited by *uvrD* and *helD* helicase mutants. The UvrABC excision repair pathway, which is responsible for efficient removal of pyrimidine dimers in UV-irradiated cells, is known to involve helicase II (37). This enzyme is directly responsible for turnover of UvrC after dyad incision on either side of the DNA adduct (32). The role of helicase IV in the repair of UV dimers has never been explored. To determine the UV resistance phenotype of a cell strain containing the *helD* deletion and to understand the relationship which exists between helicases II and IV with regard to the repair of UV lesions, the UV resistance phenotype of each of the single and double mutants was examined (Fig. 6). The parental wild-type strain, GE1752, demonstrated 100% viability regardless of the UV fluence utilized in this experiment. Approximately 100% of the GEΔH4 cells were also found to be viable after exposure to a UV fluence of 21 J/m^2, suggesting that UV damage repair in *E. coli* does not directly involve helicase IV. GEΔH2 cells, as expected, were sensitive to UV irradiation. After exposure to a fluence of 21 J/m^2, only 0.3% of the cells remained viable. The UV sensitivity of GEΔH2 is not strongly influenced by the presence of a *helD* deletion, suggesting that, in the absence of helicase II, there is no direct involvement of helicase IV in any UV repair mechanism.

MMS, like several other alkylating agents, brings about base modifications in the DNA resulting in toxic lesions (13). Removal of the alkylated bases results in the formation of a highly mutagenic apurinic or apyrimidinic site. Helicase II has previously been shown to be required for the efficient repair of DNA damage caused by alkylating agents (35). Recent findings (48) have shown a Δ*uvrD* strain to have slight MMS sensitivity, compared with the highly sensitive phenotype of a *uvrD3* mutant (36), suggesting that some other function associated with the *uvrD3* protein is responsible for MMS sensitivity. The involvement of helicase IV in the repair of alkylated DNA has never been investigated. To elucidate the role of helicase IV and to confirm the recent findings involving helicase II, the survival of both the single- and double-deletion mutants in the presence of MMS was analyzed. As shown in Fig. 7, GEΔH4 exhibited a slightly greater resistance to MMS than the parent strain GE1721. This effect was reproducible. GEΔH2, as expected, was slightly more sensitive to MMS than the parental strain. Qualitatively similar results were seen in a JC7623 strain background (data not shown).

Effect of *helD* and *uvrD* on recombination. Genetic studies have so far linked 16 genes with the process of recombination in *E. coli* K-12 (9, 39). These genes have been identified by decreases in the recombination efficiency of the mutant strains in different genetic backgrounds (21, 25). Helicase II has been shown to be involved in the RecF pathway of recombination (15), and helicase IV has been implicated in the same pathway (25a). To further investigate the involvement of the two helicases in recombination, the Δ*helD*::*cam* and the Δ*uvrD*::*tet* alleles were introduced into appropriate

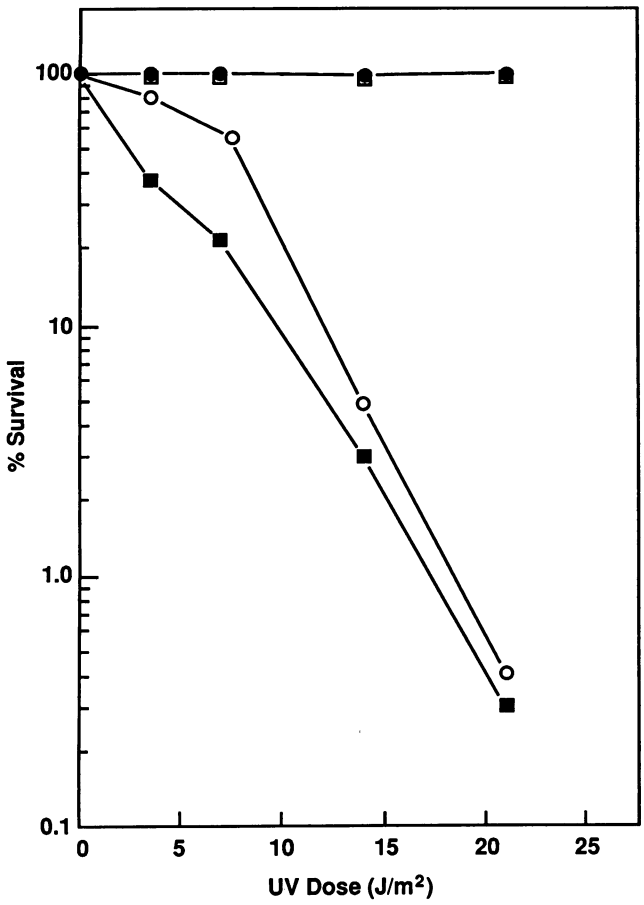

FIG. 6. UV sensitivity of the deletion strains. Stationary cells were exposed to UV light (254 nm) at the indicated fluence, plated onto Luria-Bertani agar, and incubated at 37°C for 24 h. Data (percent survival) are expressed as the number of UV-irradiated cells forming colonies as a fraction of the colonies formed by unirradiated cells. ●, GE1752 (GE1721) (*uvrD*$^+$ *helD*$^+$); □, GEΔH4 (*uvrD*$^+$ Δ*helD*::*cam*); ■, GEΔH2 (Δ*uvrD*::*tet helD*$^+$); ○, GEΔH2ΔH4 (Δ*uvrD*::*tet* Δ*helD*::*cam*).

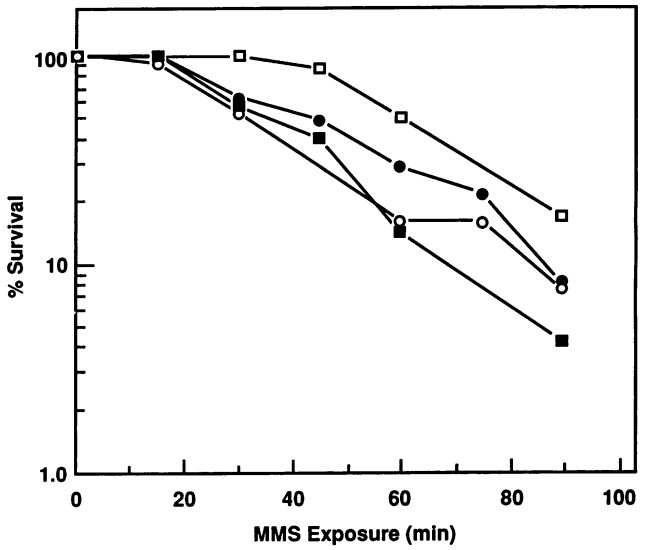

FIG. 7. MMS sensitivity of the deletion strains. Stationary cells were exposed to 2.4×10^{-2} M MMS for the time period indicated and then, after neutralization of MMS, were plated on Luria-Bertani agar and incubated at 37°C for 24 h. Data (percent survival) are expressed as the number of MMS-exposed cells forming colonies as a fraction of colonies formed by unexposed cells. ●, GE1752 (GE1721) (*uvrD*$^+$ *helD*$^+$); □, GEΔH4 (*uvrD*$^+$ Δ*helD*::*cam*); ■, GEΔH2 (Δ*uvrD*::*tet helD*$^+$); ○, GEΔH2ΔH4 (Δ*uvrD*::*tet* Δ*helD*::*cam*).

genetic backgrounds (Table 1). Each of the mutant strains was examined for conjugational and transductional recombination proficiency. For conjugational recombination, as shown in Table 3, the Δ*helD* allele caused a marginal increase in recombination proficiency in a *recBC*$^+$ background and a modest decrease in recombination proficiency in a *recBC sbcB*(*C*) background. A similar decrease is seen in the recombination proficiency of a *uvrD* deletion in the *recBC sbcB*(*C*) background, which is consistent with earlier observations (15). It should be noted that deletion of the *uvrD* gene from the genome considerably lowered the viability of this cell strain (Table 3), which partially contributed to the decrease in conjugational recombination frequency. The previously reported hyper-rec phenotype (3) for *uvrD* mutations was not observed in the AB1157ΔH2 (*recBC*$^+$ Δ*uvrD*::*tet*) strain studied. This could be due to either differences in strain backgrounds or the specific *uvrD* alleles used in the studies. In cell strains with an operating RecE pathway of recombination (*recBC sbcA*), both the *helD* and the *uvrD* deletions decreased recombination frequency approximately 10-fold when compared with the parent, JC8679.

A more profound effect on conjugational recombination efficiency was exhibited by the double Δ*helD*-Δ*uvrD* deletion in the *recBC sbcB*(*C*) cell strain background. In this case, the conjugational recombination efficiency was reduced 100-fold or more (Table 3). In the *recBC sbcA* background, the double-deletion mutant exhibited a 10-fold decrease in conjugational recombination efficiency similar to either of the single-deletion mutants. The decrease in the efficiency of conjugational recombination was specific for the *recBC sbcB*(*C*) and the *recBC sbcA* backgrounds and was not detected in the wild-type *recBC*$^+$ *sbc*$^+$ background. In the latter, the double-deletion mutant exhibited only a modest decrease in recombination. Similar defects in recombination efficiency were also observed in the RecF and the RecE pathways during transductional recombination (Table 3).

DISCUSSION

Both a *helD* deletion and a *uvrD* deletion have been successfully introduced into *E. coli* by gene replacement techniques (18, 19). In each case, the deleted gene has been marked with an antibiotic resistance gene. The construction of single and double deletions of helicases II and IV has shown that neither of these helicases is required for cell viability. In the case of helicase II, this confirms results previously reported (48). This suggests that neither of these helicases acts in a pathway essential for cell viability. Alternatively, the absence of either helicase might be compensated for by the presence of another helicase with similar biochemical properties.

As expected, cells lacking helicase II exhibited an increased sensitivity to UV irradiation. The presence of a *helD* deletion did not increase or decrease the UV sensitivity. However, Δ*uvrD*-Δ*rep* cells exhibit a slightly higher resistance to UV irradiation than the Δ*uvrD* strain. This in-

TABLE 3. Effects of Δ*helD* and Δ*uvrD* on conjugational and transductional recombination[a]

Strain	Relevant genotype	Viability[b]	Relative yield of Thr$^+$ Leu$^+$ transconjugants	Recombination efficiency[c]	Relative yield of Leu$^+$ transductants[d]
AB1157[e]	*rec*$^+$	1.00 (2.2 × 10^8cells/ml)	1.00 (8.56 × 10^6cells/ml)	1.00	ND[f]
AB2463	*recA13*	1.00	0.00	0.00	0.00
AB1157ΔH2	Δ*uvrD*	0.46	0.14	0.30	ND
AB1157ΔH4	Δ*helD*	1.53	2.23	1.46	ND
AB1157ΔH2ΔH4	Δ*uvrD* Δ*helD*	0.62	0.20	0.32	ND
JC7623	*recBC sbcB*(*C*)	0.54	0.52	0.96	1.00
JC7623ΔH2[g]	*recBC sbcB*(*C*) Δ*uvrD*	0.22	0.07	0.32	0.45
JC7623ΔH4	*recBC sbcB*(*C*) Δ*helD*	0.75	0.42	0.56	0.47
JC7623ΔH2ΔH4	*recBC sbcB*(*C*) Δ*uvrD* Δ*helD*	0.45	0.0024	0.0053	0.011
JC8679	*recBC sbcA*	0.71	0.27	0.38	0.83
JC8679ΔH2	*recBC sbcA* Δ*helD*	0.35	0.02	0.057	0.18
JC8679ΔH4	*recBC sbcA* Δ*uvrD*	0.46	0.03	0.065	0.47
JC8679ΔH2ΔH4	*recBC sbcA* Δ*uvrD* Δ*helD*	0.22	0.0046	0.021	0.16

[a] Matings were performed in Luria-Bertani broth at 37°C for 60 min with donor Hfr JC158 and recipient cultures grown to an A_{600} of 0.4 (approximately 2 × 10^8 cells per ml as determined by viable count) before mixing. The transconjugant values given are relative to those for AB1157 strains mated in parallel (see Materials and Methods) and are the means from at least two (usually four to six) independent sets of experiments. The standard deviations in most cases were between 5 and 15% of the means. The actual values determined for the controls were 2.2 × 10^8 viable cells and 8.56 × 10^6 transconjugants per ml of the mating mixture.

[b] Measures of viability relate to the number of CFU in the recipient cultures at an A_{600} of 0.4 (see Materials and Methods). Standard deviations for viability (unless stated otherwise) ranged from 3 to 20% of the mean and are excluded here for clarity of presentation.

[c] Recombination efficiency is relative yield of transconjugants divided by viability and is included to normalize the differences in viability.

[d] The P1 donor of *leu*$^+$ was JC158. Values given are relative to JC7623, which yielded 3 × 10^{-5} to 4 × 10^{-5} Leu$^+$ transductants per PFU in the lysate in all determinations. The reported relative yield of transductants has been normalized for viability differences. All strains analyzed for transductions have been verified for P1 infectivity.

[e] All of the strains used for conjugational recombination are derivatives of AB1157.

[f] ND, not determined.

[g] The standard deviation for viability was greater than 50% of the mean, because the cells had a poor viability and a low growth rate.

creased UV resistance is lost upon deleting *helD* (19a). This suggests that in the absence of both Rep protein and helicase II, helicase IV may be either directly or indirectly (through an inducible pathway) involved in eliminating a small fraction of pyrimidine dimers. The introduction of Δ*helD* into a wild-type strain slightly, but reproducibly, increased the resistance of the mutant strains to MMS. Perhaps the absence of helicase IV triggers an inducible repair pathway or allows proper functioning of a constitutive repair pathway involved in the repair of alkylated bases.

Historically, *E. coli* recombination genes have been classified as acting in the RecBC, RecF, or RecE recombination pathway, depending on whether they were required for conjugational recombination in the wild-type, *recBC sbcB* (*C*), or *recBC sbcA* background, respectively (9). Previous genetic studies have implicated helicase II in the RecF pathway of recombination (15). As deduced from the studies presented here, *helD* and *uvrD* together behave as genes typically involved in the RecF pathway of recombination. Deletion of either one or both genes has little effect on recombination in a wild-type background (*recBC*$^+$ *sbcBC*$^+$). However, the double-deletion mutant exhibits a profound effect on recombination in the *recBC sbcB*(*C*) background, in which the RecBC pathway has been disabled, and most recombination is thought to proceed via the RecF pathway (9). In the *recBC sbcA* background, where most of the recombination is thought to occur via the RecE pathway, the double *uvrD-helD* deletion mutants show no greater effect than either of the single-deletion mutants. This differential effect of the double *uvrD-helD* deletion mutants on the RecF and the RecE pathways suggests that the requirement for helicases II and IV may be less stringent in the *recBC sbcA* background, probably reflecting the different roles played by the helicases in the two recombination pathways. For example, in the *recBC sbcB*(*C*) background, either helicase II or helicase IV is required for some aspect of conjugational or transductional recombination. The modest decrease observed in the recombination frequencies of the single-deletion mutants suggests that helicases II and IV can apparently compensate for each other. With regard to the *recBC sbcA* background, the two helicases seem to function at two different stages in the same recombination pathway. Hence, eliminating either of the helicases affects recombination 10-fold, with the double-deletion mutants showing no greater effect than either of the single-deletion mutants. Such a differential effect on recombination in the RecF and the RecE backgrounds has also been observed for *ruv* mutants (5) and certain alleles of *recJ* (26).

DNA helicase involvement in recombination has been suggested for both the presynaptic and the postsynaptic events of recombination. A common theme for presynaptic involvement suggests that a combination of helicase and nuclease activities could provide the single-stranded DNA substrate required by the RecA protein (9). Such a coupling of activities has been suggested for the RecQ helicase and the RecJ nuclease in the RecF recombination pathway (47). Functionally, RecQ protein is very similar to both helicase II and helicase IV (47), and the involvement of more than one helicase in a recombination pathway is plausible.

Helicase action has also been implicated in postsynaptic strand exchange. In this case, the enzyme may be required either for paranemic to plectonemic joint conversion or for branch migration (9). The latter has been observed in the case of RuvAB, where RuvA acts as the specificity factor and the RuvB ATPase acts as the motor for driving energy-efficient branch migration (46). However, further experimentation involving helicase mutants in different genetic backgrounds with different recombination substrates

must be completed to obtain a more detailed understanding of helicase involvement in recombination and repair of DNA.

ACKNOWLEDGMENTS

We thank Barbara Bachmann, Cathy Joyce, Stan Tabor, and George Weinstock for their generous gifts of bacterial and viral strains and plasmids; Jim George for stimulating discussions; and Susan Whitefield for artwork. We are grateful to Kenneth N. Kreuzer for critically reading the manuscript.

This investigation was supported by grant GM33476 from the National Institutes of Health to S.W.M.

REFERENCES

1. **Abdel-Monem, M., G. Taucher-Schloz, and M. Q. Klinkert.** 1983. Identification of *Escherichia coli* DNA helicase I as the *traI* gene product of the F factor. Proc. Natl. Acad. Sci. USA **80**:4659–4663.
2. **Appleyard, R. K.** 1954. Segregation of new lysogenic types during growth of a doubly lysogenic strain derived from *Escherichia coli* K12. Genetics **39**:440–452.
3. **Arthur, H. M., and R. G. Lloyd.** 1980. Hyper-recombination in *uvrD* mutants of *Escherichia coli* K-12. Mol. Gen. Genet. **180**:185–191.
4. **Bachmann, B. J.** 1987. Linkage map of *Escherichia coli* K-12, p. 807–876. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. American Society for Microbiology, Washington, D.C.
5. **Benson, F., S. Collier, and R. G. Lloyd.** 1991. Evidence of abortive recombinants in *ruv* mutants of *Escherichia coli* K-12. Mol. Gen. Genet. **225**:266–272.
6. **Bolivar, F., R. L. Rodrigues, P. J. Greene, M. C. Betlach, and H. L. Heyneker.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
7. **Boyer, H. W., and D. Roulland-Dussoix.** 1969. A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41**:459–472.
8. **Cameron, J. R., S. M. Panasenko, I. R. Lehman, and R. W. Davis.** 1975. *In vitro* construction of bacteriophage λ carrying segments of the *Escherichia coli* chromosome: selection of hybrids containing the gene for DNA ligase. Proc. Natl. Acad. Sci. USA **72**:3416–3420.
9. **Clark, A. J.** 1991. *rec* genes and homologous recombination proteins in *Escherichia coli*. Biochimie **73**:523–532.
10. **Clark, A. J., and A. D. Marguiles.** 1965. Isolation and characterization of recombination deficient mutants of *E. coli* K12. Proc. Natl. Acad. Sci. USA **53**:451–459.
11. **Cohen, A., and A. J. Clark.** 1986. Synthesis of linear plasmid multimers in *Escherichia coli* K-12. J. Bacteriol. **167**:327–335.
12. **Davis, L. G., M. D. Dibner, and J. F. Battery.** 1986. Basic methods in molecular biology. Elsevier Science Publishing Co., New York.
13. **Freidberg, E. C.** 1985. DNA repair. Freeman & Co., New York.
14. **Gillen, J. R., D. K. Willis, and A. J. Clark.** 1981. Genetic analysis of the RecE pathway of genetic recombination in *Escherichia coli* K-12. J. Bacteriol. **145**:521–532.
15. **Horii, Z.-I., and A. J. Clark.** 1973. Genetic analysis of the RecF pathway to genetic recombination in *Escherichia coli* K-12: isolation and characterization of mutants. J. Mol. Biol. **80**:327–344.
16. **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.
17. **Howard-Flanders, P., and L. Theriot.** 1966. Mutants of *Escherichia coli* K-12 defective in DNA repair and in genetic recombination. Genetics **53**:1137–1150.
18. **Jasin, M., and P. Schimmel.** 1984. Deletion of an essential gene in *Escherichia coli* by site-specific recombination with linear DNA fragments. J. Bacteriol. **159**:783–786.
19. **Joyce, C. M., and N. D. F. Grindley.** 1984. Method for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. J. Bacteriol. **158**:636–643.

19a. **Kaiser-Rogers, K., and S. W. Matson.** Unpublished observations.

20. **Kohara, Y., A. Kiyotaka, and I. Katsumi.** 1987. The physical map of the whole *E. coli* chromosome: application of a new strategy for rapid analysis and sorting of a large genomic library. Cell **50**:495–508.
21. **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.
22. **Kunkel, T. A., J. B. Roberts, and R. A. Zakour.** 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154**:367–382.
23. **LeBowitz, J. H., and R. McMacken.** 1986. The *Escherichia coli* DnaB replication protein is a DNA helicase. J. Biol. Chem. **261**:4738–4748.
24. **Lee, M. S., and K. Marians.** 1987. *Escherichia coli* replication factor Y, a component of the primosome, can act as a DNA helicase. Proc. Natl. Acad. Sci. USA **84**:8345–8352.
25. **Lloyd, R. G., M. C. Porton, and C. Buckman.** 1988. Effect of *recF*, *recJ*, *recN*, *recO* and *ruv* mutations on ultraviolet survival and genetic recombination in a *recD* strain of *Escherichia coli* K-12. Mol. Gen. Genet. **212**:317–324.

25a. **Lovett, S.** Personal communication.

26. **Lovett, S., and A. J. Clark.** 1984. Genetic analysis of the *recJ* gene of *Escherichia coli* K-12. J. Bacteriol. **157**:190–196.
27. **Matson, S. W.** 1991. DNA helicases of *E. coli*. Prog. Nucleic Acid Res. Mol. Biol. **40**:289–326.
28. **Matson, S. W., and K. A. Kaiser-Rogers.** 1990. DNA helicases. Annu. Rev. Biochem. **59**:289–329.
29. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
30. **Modrich, P.** 1989. Methyl-directed DNA mismatch correction. J. Biol. Chem. **264**:6597–6600.
31. **Oh, E. Y., and L. Grossman.** 1987. Helicase properties of the *Escherichia coli* UvrAB protein complex. Proc. Natl. Acad. Sci. USA **84**:3638–3642.
32. **Orren, D. K., C. P. Selby, J. E. Hearst, and A. Sancar.** 1992. Post-incision steps of nucleotide excision repair in *Escherichia coli*. J. Biol. Chem. **267**:780–788.
33. **Sambrook, J., E. F. Fritsch, and R. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Scott, J. F., and A. Kornberg.** 1978. Purification of the Rep protein of *Escherichia coli*. J. Biol. Chem. **253**:3292–3297.
35. **Seigel, E. C.** 1973. Ultraviolet-sensitive mutator strain of *Escherichia coli* K-12. J. Bacteriol. **113**:145–160.
36. **Seigel, E. C., and H. M. Race.** 1981. Phenotypes of UV-sensitive *uvrD3*, *recL152*, and *uvrE15* mutants of *Escherichia coli*. Mutat. Res. **83**:49–59.
37. **Selby, C. P., and A. Sancar.** 1988. ABC excinuclease incises both 5′ and 3′ to the CC-1065-DNA adduct and its incision activity is stimulated by DNA helicase II and DNA polymerase I. Biochemistry **27**:7184–7188.
38. **Short, J. M., J. M. Fernandez, J. A. Sorge, and W. D. Huse.** 1988. λ ZAP: a bacteriophage λ expression vector with *in vivo* excision properties. Nucleic Acids Res. **16**:7583–7600.
39. **Smith, G. R.** 1989. Homologous recombination in *E. coli*: multiple pathways for multiple reasons. Cell **58**:807–809.
40. **Studier, F. W., and B. A. Moffat.** 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. **189**:113–130.
41. **Studier, F. W., A. H. Rosenberg, J. J. Dunn, and J. W. Dubendorff.** 1990. Use of T7 RNA polymerase to direct expression of cloned genes. Methods Enzymol. **185**:60–89.
42. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82**:1074–1078.
43. **Taucher-Scholz, G., and H. Hoffmann-Berling.** 1983. Identifica-

tion of the gene for DNA helicase II of *Escherichia coli*. Eur. J. Biochem. **137**:573–580.
44. **Taylor, A., and G. R. Smith.** 1980. Unwinding and rewinding of the DNA by the RecBC enzyme. Cell **22**:447–457.
45. **Trun, N. J., and T. J. Silhavy.** 1987. Characterization and *in vivo* cloning of *prlC*, a suppressor of signal sequence mutations in *Escherichia coli* K12. Genetics **116**:513–521.
46. **Tsaneva, I. R., B. Muller, and S. C. West.** 1992. ATP-dependent branch migration of Holliday junctions promoted by the RuvA and RuvB proteins of *E. coli*. Cell **69**:1171–1180.
47. **Umezi, K., K. Nakayama, and H. Nakayama.** 1990. *Escherichia coli* RecQ protein is a DNA helicase. Proc. Natl. Acad. Sci. USA **878**:5363–5367.
48. **Washburn, B. K., and S. R. Kushner.** 1991. Construction and analysis of deletions in the structural gene (*uvrD*) for DNA helicase II of *Escherichia coli*. J. Bacteriol. **173**:2569–2575.
49. **Willetts, N. S., A. J. Clark, and B. Low.** 1969. Genetic location of certain mutations conferring recombination deficiency in *Escherichia coli*. J. Bacteriol. **97**:244–249.
50. **Wilson, K.** 1989. Preparation of genomic DNA from bacteria, p. 2.4.1–2.4.5. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
51. **Wood, E. R., and S. W. Matson.** 1987. Purification and characterization of a new DNA-dependent ATPase with helicase activity from *Escherichia coli*. J. Biol. Chem. **262**:15269–15276.
52. **Wood, E. R., and S. W. Matson.** 1989. The molecular cloning of the gene encoding the *Escherichia coli* 75-kDa helicase and the determination of its nucleotide sequence and genetic map position. J. Biol. Chem. **262**:8297–8303.
52a. **Wood, E. R., and S. W. Matson.** Unpublished observations.
53. **Yarronton, G. T., R. H. Das, and M. L. Gefter.** 1979. Enzyme-catalysed DNA unwinding. J. Biol. Chem. **254**:11997–12001.