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UvrD Helicase Suppresses Recombination and DNA Damage-Induced Deletions†

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UvrD, a highly conserved helicase involved in mismatch repair, nucleotide excision repair (NER), and recombinational repair, plays a critical role in maintaining genomic stability and facilitating DNA lesion repair in many prokaryotic species. In this report, we focus on the UvrD homolog in *Helicobacter pylori*, a genetically diverse organism that lacks many known DNA repair proteins, including those involved in mismatch repair and recombinational repair, and that is noted for high levels of inter- and intragenomic recombination and mutation. *H. pylori* contains numerous DNA repeats in its compact genome and inhabits an environment rich in DNA-damaging agents that can lead to increased rearrangements between such repeats. We find that *H. pylori* UvrD functions to repair DNA damage and limit homologous recombination and DNA damage-induced genomic rearrangements between DNA repeats. Our results suggest that UvrD and other NER pathway proteins play a prominent role in maintaining genome integrity, especially after DNA damage; thus, NER may be especially critical in organisms such as *H. pylori* that face high-level genotoxic stress in vivo.

Genomic alterations due to recombination, deletions, and duplications are a major source of genetic plasticity in all prokaryotes (14, 15). To minimize such damage, bacteria have evolved mechanisms involving mismatch repair (MMR) proteins such as MutSLH, nucleotide excision repair (NER) proteins such as UvrABCD, and recombinational repair proteins such as RecBCD and RecFOR (32, 45, 55, 66). *Helicobacter pylori*, a gram-negative gastric bacterium found in humans, is unusual because it lacks many of the genes of major recognized DNA repair and recombination pathways, including *mutSLH*, *recCD*, and *recFO*, common to other bacteria (5, 60). *H. pylori* has a high rate of recombination and mutation, with *H. pylori* variants arising over relatively short time periods (months) in individual hosts (1, 17, 56). As essentially the only persistent bacterium in its niche, the high level of genetic diversity in *H. pylori* may facilitate adaptation to its environment through the continual generation of variants and selection for highest fitness (65).

H. pylori possesses a homolog of the DNA repair helicase, UvrD (annotated as HP1478 in sequenced strain 26695 and as JHP1371 in sequenced strain J99) (5, 60). In *Escherichia coli*, UvrD works in concert with MutSHL in the MMR pathway and with UvrABC in the NER system (Fig. 1). Disruption of MMR leads to increased spontaneous mutations and homologous recombination (22, 51), whereas disruption of NER increases sensitivity to UV and other agents of DNA damage (12, 33). UvrD also has an important role in restarting stalled replication forks and facilitates this process by displacing RecA protein from DNA (20) (Fig. 1).

We asked what role a *uvrD* homolog would play in an organism such as *H. pylori*, which is highly diverse and lacks known repair proteins, including MutSHL. Would *H. pylori* UvrD limit illegitimate recombination and mutation in the absence of MMR?

As in other bacteria with small genomes, the *H. pylori* chromosome possesses a large number of direct, nonrandomly distributed repeats throughout its genome that can promote genomic rearrangements (7). Previous studies have shown that a helicase, RecG, functions to limit deletions between such repeats (31). We asked whether the UvrD helicase would likewise influence deletions. *H. pylori* resides in the human gastric mucosa, where it is exposed to various sources of DNA damage, including oxidative radicals and nitrosative species (9, 13, 46). Therefore, we also explored the effect of *uvrD* mutation on inter- and intragenomic recombination after exposure to DNA damage.

Our studies demonstrate that *H. pylori* UvrD, like its *E. coli* homolog, plays an important role in repairing damaged DNA. We find that UvrD has no effect on spontaneous mutation but limits homologous intergenomic recombination in *H. pylori* despite the absence of MutSHL, indicating UvrD functional independence from MutSHL in this regard. In contrast to the RecG helicase, UvrD does not influence deletions between *H. pylori* DNA repeats under usual in vitro conditions, but with DNA damage, deletion frequency is significantly increased in the UvrD mutant. These findings indicate important roles for *H. pylori* UvrD in maintaining genomic integrity in the stressful environments in which it normally resides in these organisms.

MATERIALS AND METHODS

Statistical analyses. Student's *t* test was used where appropriate, with a P value < 0.05 deemed significant.

Phylogeny and amino acid alignment of UvrD homologs. GenBank (www.ncbi.nih.gov/GenBank/) was searched for homologs to *H. pylori* HP1478 by use of the BLAST-p algorithm (41). Alignments of amino acid sequences were constructed using the program ClustalX and the Gonnet 250 protein

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† Supplemental material for this article may be found at http://jb.asm.org/.

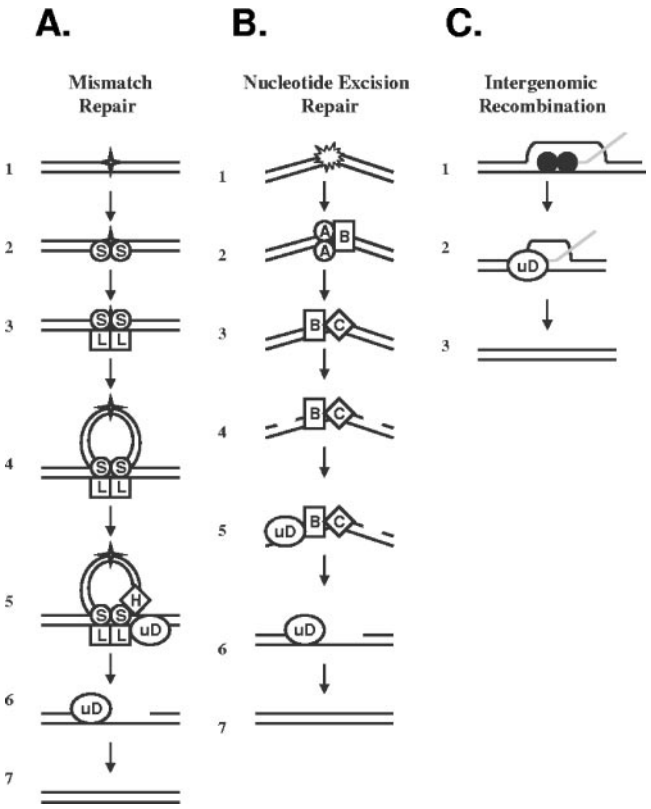


FIG. 1. Proposed mechanisms for UvrD in mismatch repair, nucleotide excision repair, and intergenomic recombination. (A) In *E. coli*, the MMR pathway corrects mismatches, small insertions, and deletions (represented by the star) (row 1). The MutS1 dimer recognizes the mismatch (row 2) and recruits MutL (row 3). The MutS1-MutL complex translocates along the DNA, forming a loop (row 4). MutH endonuclease nicks the DNA (row 5). UvrD helicase, which interacts physically with MutL (23), is recruited to the lesion and removes it (row 5), leaving a gap (row 6) which is filled in by DNA polymerase III, restoring the template (row 7). (B) The NER pathway repairs a variety of bulky lesions, including pyrimidine dimers and alkylated base pairs, symbolized in row 1. The lesion is recognized by UvrA, which recruits UvrB helicase (row 2). UvrA subsequently detaches, and UvrC endonuclease is recruited to the lesion (row 3). The UvrBC complex nicks the DNA 5′ and 3′ of the lesion (row 4). UvrD helicase, which interacts physically with UvrB (2), is recruited to the site (row 5) and removes the lesion, leaving a gap (row 6) that subsequently is filled by DNA polymerase I and restored (row 7). (C) In intergenomic recombination, strand invasion is mediated by RecA (filled circles) (row 1). The UvrD helicase dismantles the RecA-DNA complex (row 2) (20, 44, 63), leading to restoration of the template (row 3).

weight matrix (57) and visualized using the program Genedoc (www.psc.edu/biomed/genedoc) in three-shading mode. A phylogenetic tree was constructed using the program Mega 3 (34) and the neighbor-joining method (52) with 1,000 bootstrap replicates.

Bacterial strains and plasmids. The *E. coli* and *H. pylori* strains and plasmids used in this study are listed in Table 1. Wild-type *E. coli* strain JJC40 (10) and its isogenic *uvrD* mutant, JJC212, kindly provided by Benedicte Michel, were routinely grown in Luria-Bertani broth (LB) at 37°C. *H. pylori* strains were routinely grown at 37°C in 5% CO_2 on Trypticase soy agar (TSA) plates or brucella agar (BA) with 10% newborn calf serum (NCS) plates supplemented with the appropriate antibiotic.

Plasmid pECuD was constructed using primers ECUDXbaI and ECUDSmaI and plasmid pHPuD was constructed using primers HPUDXbaI and HPUDSmaI as described previously (30, 31) (see Table S2 in the supplemental material). The features of all recombinant plasmids were confirmed by PCR.

Construction of *H. pylori* mutants used to assess susceptibility to UV, intergenomic recombination frequencies, and spontaneous mutation frequencies. Fragments of HP1478 (*uvrD* homolog), HP0887 (*vacA*), and HP0153 (*recA*) open reading frames (ORFs) were amplified by PCR using primers based on sequenced strain 26695 (see Table S2 in the supplemental material) and cloned into pGEMT-Easy (Promega, Wisconsin) to create pUvrD, pVacA, and pRecA, respectively (31). The *aphA* cassette, conferring kanamycin resistance (Kan^r), was used to interrupt each ORF to create pUvrDKm, pVacAKm, and pRecAKm, respectively. The *vacA* locus was chosen as a control for the presence of the *aphA* cassette, since it is not involved in recombination and its construction has been documented previously (7, 31). The *recA* locus was also interrupted, since several phenotypes that are related to this study have been observed in mutants (7, 31, 54, 58). *H. pylori* strain JP26 was transformed to Kan^r with pUvrDKm, pVacAKm, and pRecAKm to create JP26 *uvrD*::*aphA*, *vacA*::*aphA*, and *recA*::*aphA*, respectively, as described previously (64). Chromosomal DNA was isolated from transformants, and the correct insertion of the *aphA* cassette into the expected ORF was confirmed by PCR in each case.

Construction of *H. pylori* mutants used to assess deletion frequencies. A unique BamHI site was created in pUvrD and pVacA by use of inverse PCR and primers UDinvR and UDinvF based on sequenced strain 26695 (see Table S2 in the supplemental material), and each ORF was subsequently interrupted with a deletion cassette (see Fig. 4B) containing either 0-, 50-, or 100-bp repeats to create pUvrD-0, pUvrD-50, and pUvrD-100 and pVacA-0, pVacA-50, and pVacA-100. The construction and use of the deletion cassettes and JP26 *uvrB*::*100* and *recA*::*100* strains has been previously described (7). *H. pylori* strain JP26 was subsequently transformed to chloramphenicol resistance (Cat^r) with these plasmids to create JP26 *uvrD*::0, *uvrD*::50, and *uvrD*::100 and JP26 *vacA*::0, *vacA*::50, and *vacA*::100. Chromosomal DNA was isolated from transformants, and the correct insertion of the deletion cassette into the expected ORF was confirmed by PCR in each case.

Spontaneous mutation rate assay to assess mismatch repair function. Since a point mutation in *rpoB* confers resistance to rifampin in *H. pylori* (25) as well as in *E. coli*, rifampin resistance can be used as a phenotype to calculate spontaneous mutation rates. Nine colonies of each strain to be tested were picked and expanded to TSA plates. After 48 h growth at 37°C in 5% CO_2, the bacteria were suspended in 1 ml Brucella broth and serially diluted onto TSA plates alone or onto rifampin plates. *H. pylori* strains were plated on BA plates supplemented with rifampin (7.5 ug/ml) and 10% NCS; *E. coli* strains were plated on LB plates supplemented with rifampin (50 ug/ml). Mutation rates were determined from frequencies based on the Lea-Coulson algorithm (35).

Recovery from DNA damage. *E. coli* or *H. pylori* cells to be tested were grown on TSA plates for 24 h or 48 h, respectively, and suspended in 1 ml phosphate-buffered saline (PBS). Equal amounts of suspension were inoculated on TSA plates at dilutions that would produce 100 to 500 CFU per plate postexposure. Cells then were exposed to 0 to 1,400 kJ/m^2 UV at 312 nm (Stratagene Transluminator, La Jolla, CA) and incubated at 37°C, and colonies were counted and survival rates calculated.

Streptomycin resistance frequency assay to assess intergenomic recombination. *H. pylori* strains were grown on TSA plates for 48 h and harvested into 1 ml of PBS, and 25 μl was spotted onto a fresh TSA plate combined with 30 ng of donor DNA and incubated for 18 h at 37°C in 5% CO_2. Donor DNA was an 800 bp PCR product of *H. pylori rpsL* from streptomycin-resistant (St^r) strain JP26 with A128G (61). The transformation mixture was then harvested into 1 ml PBS, and 100 μl of the appropriate serial dilutions was plated onto either TSA or BA plates containing 10% NCS and 25 μg/μl streptomycin. The plates were incubated for 4 days at 37°C in 5% CO_2, and the total recombination frequency was determined by calculating the number of St^r colonies divided by the total number of CFU. As a negative control, *H. pylori* strains with no DNA added were also tested in parallel in each experiment; no colonies were seen in any case. To assess the influence of DNA damage on intergenomic recombination, *H. pylori* strains to be tested were incubated at 37°C in a 5% CO_2 environment for 48 h and subsequently exposed to 0 to 1,400 kJ/m^2 UV irradiation at 312 nm, and then assays were performed as described above.

Deletion frequency assays in *H. pylori*. To assess recombination frequencies in the *H. pylori* strains containing the deletion cassette or control cassettes, the cells were grown on TSA plates for 48 h at 37°C (5% CO_2), allowing for deletions to occur, and then harvested and washed twice in PBS, and 25, 100, and 200 μl aliquots were spread on BA plates supplemented with NCS and 25 μg/ml kanamycin. As further controls, 200 μl from each suspension was inoculated to BA plates containing NCS, kanamycin (25 μg/ml), and chloramphenicol (20 μg/ml); as expected, in no experiments were strains with double resistance identified, confirming the specificity of the deletion process (7). Total CFU and numbers of Kan^r deletion mutants were determined by plating serial dilutions onto TSA plates and BA plates with kanamycin (25 μg/ml), respectively. Plates were incu-

TABLE 1. Plasmids and strains used in this study

Plasmid or strain	Relevant characteristic(s)	Source or reference
Plasmids		
pVacAKm	*vacA*::*aphA* in pGEMT-Easy	31
pRecAKm	*recA*::*aphA* in pGEMT-Easy	31
pUvrD	HP1478 in pGEMT-Easy	This work
pUvrDKm	HP1478::*aphA* in pGEMT-Easy	This work
pVacA0	*vacA* with 0 bp deletion cassette	7
pVacA50	*vacA* with 50 bp deletion cassette	7
pVacA100	*vacA* with 100 bp deletion cassette	7
pUvrD-0	HP1478 with 0 bp deletion cassette	This work
pUvrD-0	HP1478 with 50 bp deletion cassette	This work
pUvrD-100	HP1478 with 100 bp deletion cassette	This work
pAD1	*ureAB* fragment in pUC18	6
pHPuD	pAD1 with HP1478 and CAT cassette	This work
pECuD	pAD1 with *E. coli uvrD* and CAT cassette	This work
Strains		
H. pylori		
JP26	Wild-type strain	31
JP26/*vacA*::*aphA*	*vacA* with *aphA* insertion	31
JP26/*recA*::*aphA*	*recA* with *aphA* insertion	31
JP26/*uvrD*::*aphA*	HP1478 with *aphA* insertion	This work
JP26 HPuDcomp	*uvrD*::*aphA* complemented with HP1478 in *ureAB* locus	This work
JP26 ECuDcomp	*uvrD*::*aphA* complemented with *E. coli uvrD* in *ureAB* locus	This work
JP26/*vacA*::*0*	*vacA* interrupted with 0 bp deletion cassette	7
JP26/*vacA*::*50*	*vacA* interrupted with 50 bp deletion cassette	7
JP26/*vacA*::*100*	*vacA* interrupted with 100 bp deletion cassette	7
JP26/*recA*::*100*	*recA* interrupted with 100 bp deletion cassette	7
JP26/*uvrD*::*0*	HP1478 interrupted with 0 bp deletion cassette	This work
JP26/*uvrD*::*50*	HP1478 interrupted with 50 bp deletion cassette	This work
JP26/*uvrD*::*100*	HP1478 interrupted with 100 bp deletion cassette	This work
JP26/*uvrB*::*0*	HP1114 interrupted with 0 bp deletion cassette	This work
JP26/*uvrB*::*100*	HP1114 interrupted with 100 bp deletion cassette	This work
E. coli		
JJC40	Wild-type AB1157 *hsdR*	10
JJC212	JJC40 *uvrD*::*Tn5* (Kan^r)	B. Michel

bated at 37°C in a 5% CO_2 environment for 96 h, colonies were counted, and deletion frequencies were calculated.

To assess the influence of DNA damage on intragenomic recombination, *H. pylori* strains *vacA100* and *uvrD100* were passed to TSA plates and exposed to 700 kJ/m^2 UV irradiation at 312 nm, and then assays performed as described above.

Complementation of the JP26 *uvrD*::*aphA* mutant. Plasmid pHPuD, with ORF HP1478 placed downstream of the *ureAB* promoter, was constructed using primers HPUDXbaI and HPUDSmaI and used to introduce HP1478 in *trans* into the genome of mutant JP26 *uvrD*::*aphA* via natural transformation, as described previously (6, 64), to create JP26 HPuDcomp. Transformants were selected based on Cat^r, and the correct insertion of *uvrD* and flanking regions into *ureA* was confirmed by PCR of the chromosomal DNA. In parallel experiments, pECuD was used to construct JP26 ECuDcomp, exactly as described above, by use of primers ECUDXbaI and ECUDSmaI.

Complementation of *E. coli uvrD* mutant. *E. coli* strain JJC212 (*uvrD* null mutant) was transformed with pHPuD, pECuD, and pAD1 (no insert) to create strains JJC212-HPuD, JJC212-ECuD, and JJC212-AD1, respectively. For the wild-type and transformed *E. coli* strains, recovery after exposure to DNA damage was assessed using overnight cultures that were serially diluted onto TSA plates and exposed to 0 to 1,400 kJ/m^2 UV irradiation. After incubation of plates overnight at 37°C, total numbers of colonies were counted and survival rates were determined.

RESULTS

Analyses of UvrD family homologs. The *H. pylori* UvrD homolog from sequenced strain 26695 (60), annotated as HP1478 for that strain, has 35.4% identity and 57.1% similarity to *E. coli* UvrD, which has been extensively characterized for its roles in NER, MMR, and recombinational repair (for a review, see references 28 and 53). Alignment of UvrD amino acid sequences from *H. pylori* with the closely related *Campylobacter jejuni*, as well as *Pseudomonas aeruginosa* and *E. coli*, showed that all five sequences possess the conserved helicase domains, suggesting probable conservation of function (see Fig. S1A in the supplemental material).

To understand ancestral relationships, a phylogeny was constructed using UvrD sequences from 15 representative bacterial species. As expected, the *H. pylori* UvrD homologs were most closely related with each other and cluster with that from the related *C. jejuni* (see Fig. S1B in the supplemental material); interestingly, ancestral relationships were closer with the distantly related *Thermotoga* and *Aquifex* hyperthermophile species than with *Enterobacteriaceae* and related organisms.

Role of *H. pylori* UvrD in MMR. In organisms with intact MMR, including *E. coli*, *P. aeruginosa*, and *Deinococcus radiodurans*, the absence of UvrD function results in higher spontaneous mutation frequencies (42, 48, 49) resulting from failure to unwind the mismatched strand after it has been recognized (see Fig. 1). *H. pylori* lacks homologs of MutS1, MutL, and MutH, the mismatch recognition and excision proteins (5, 30, 50, 60), and therefore the *uvrD* mutant was not expected to display a mutation rate significantly different from that of the wild type unless it participated in an unrecognized

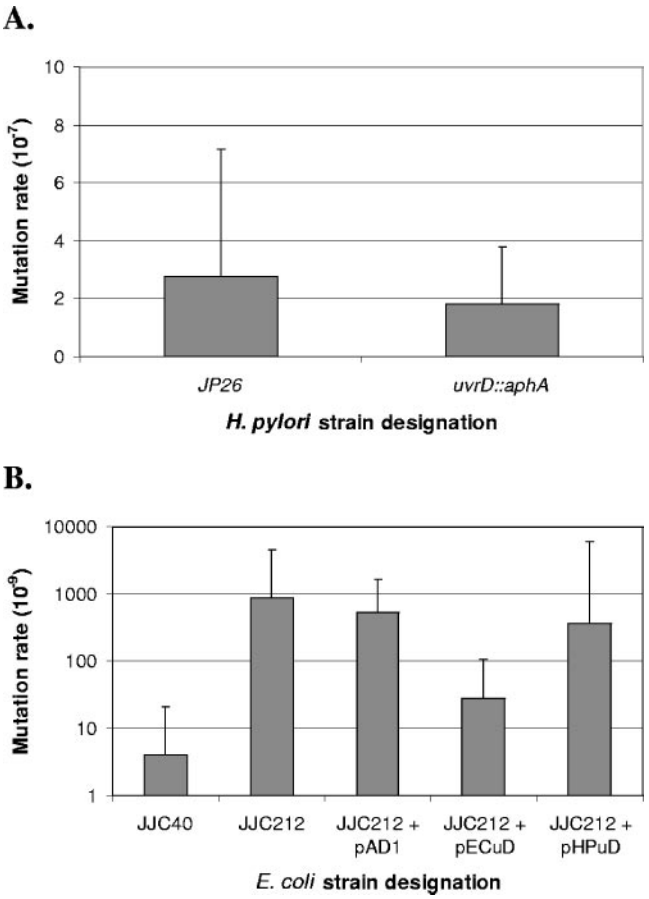


FIG. 2. Spontaneous mutation rates of *H. pylori* and *E. coli* wild-type, mutant, and complemented strains. (A) Spontaneous mutation rates of the *H. pylori* wild type (JP26) and the *uvrD* mutant were determined based on resistance to rifampin and calculated using the method of Lea-Coulson (35). Differences were not significant ($P > 0.05$). (B) Spontaneous mutation rates of *E. coli* wild-type (JJC40), *uvrD* mutant (JJC212), and *uvrD* mutant strains transformed with vector pECuD (*E. coli uvrD*), pHPuD (*H. pylori uvrD*), or pAD1 (no insert) by use of the method of Lea-Coulson. The *E. coli uvrD* mutant was partially complemented ($P < 0.05$) by *E. coli* UvrD expression but not by *H. pylori* UvrD expression ($P > 0.05$).

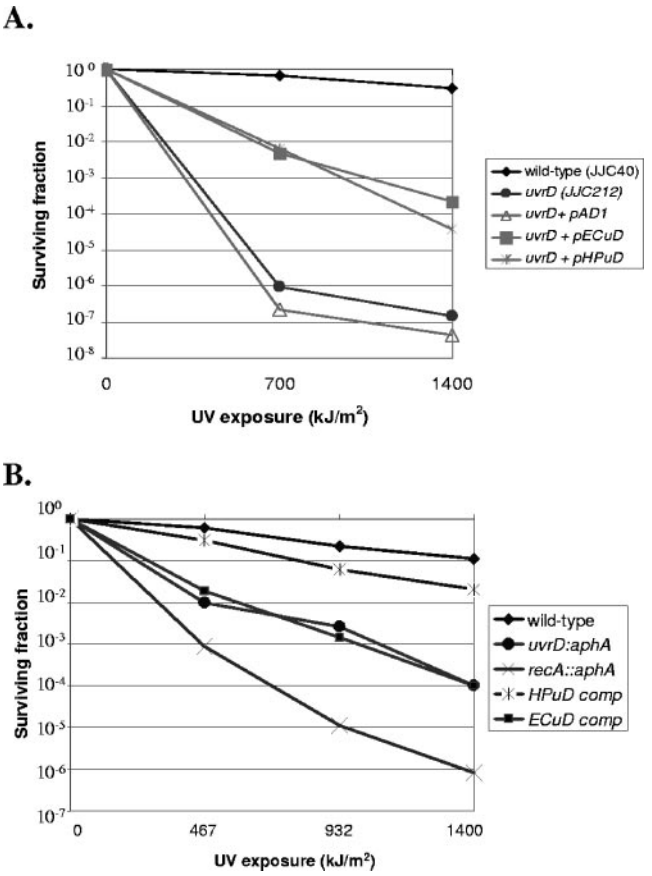


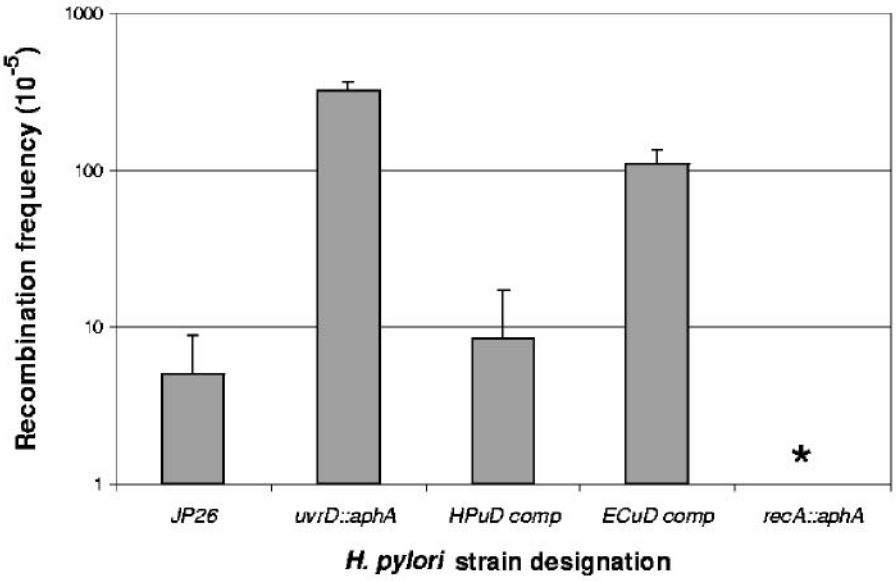
FIG. 3. Susceptibility to UV of *E. coli* and *H. pylori* wild-type, mutant, and complemented strains. (A) *E. coli* wild-type strain JJC40, *uvrD* mutant strain JJC212, and JJC212 complemented with vector pAD1 alone (no insert) or with *E. coli uvrD* in pAD1 (pECuD) or *H. pylori* HP1478 in pAD1 (pHPuD) were examined for susceptibility to UV (0 to 1,400 kJ/m^2). Results shown are representative of five experiments. Both pHPuD and pECuD complemented the *E. coli uvrD* mutant to a significant ($P < 0.05$) degree at 700 and 1,400 kJ/m^2, whereas pAD1 did not ($P > 0.05$), as expected. (B) *H. pylori* wild-type strain JP26, mutant strains JP26 *uvrD*::*aphA* and JP26 *recA*::*aphA*, and strain JP26 *uvrD*::*aphA* complemented in *trans* with an *H. pylori uvrD* homolog (HPuDcomp) or an *E. coli uvrD* homolog (ECuDcomp) were assayed for survival after exposure to UV (0 to 1,400 kJ/m^2). Results shown are representative of five experiments. As expected, the *recA*::*aphA* and *uvrD*::*aphA* strains were UV sensitive. Both *H. pylori* and *E. coli uvrD* complemented the *uvrD*::*aphA* mutant to a significant ($P < 0.05$) degree at the UV exposures tested, with the *H. pylori uvrD* complementing to a greater degree than *E. coli uvrD.*

mismatch repair pathway. We found that the absence of UvrD function in *H. pylori* does not lead to a change in mutation rates, as expected (Fig. 2A). These results also exclude the possibility of an alternative mismatch repair pathway involving UvrD.

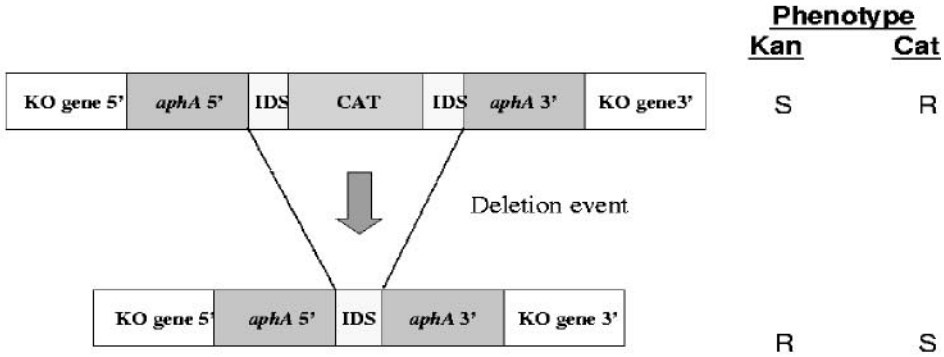
***E. coli uvrD* mutants display elevated mutation rates.** We asked whether *H. pylori* UvrD could complement this phenotype in *E. coli uvrD* mutants to determine the extent of functional similarity between *H. pylori* and *E. coli* UvrD. To address this question, JJC212, the *E. coli uvrD* mutant, was transformed with pAD1 (no insert control), pECuD (*E. coli uvrD*), or pHPuD (*H. pylori uvrD*), and mutation rates were calculated (Fig. 2B). As expected, transformation of JJC212 with pAD1 resulted in no change, whereas transformation with pECuD resulted in a partial but significant ($P < 0.01$) decrease in mutation rate. Transformation of JJC212 with pHPuD did not result in a significant degree of complementation (Fig. 2B), demonstrating that *H. pylori* UvrD was unable to complement the MMR defect in *E. coli.*

Role of *H. pylori* UvrD in nucleotide excision repair. Next, we asked whether *H. pylori* UvrD could complement the UV sensitivity phenotype of *E. coli uvrD* mutants. *E. coli* strains JJC40 and JJC212 were assayed for sensitivity to UV exposure (312 nm), and JJC212 was complemented in *trans* with pECuD or pHPuD or with pAD1 alone. As expected, JJC212 showed markedly increased susceptibility to UV damage compared to wild-type results (47), and transformation of JJC212 with pAD1 had no effect on survival (Fig. 3A). Transformation of JJC212 with either pECuD or pHPuD resulted in partial but significant complementation ($P < 0.05$ for both 700 and

A.


Recombination frequency (10^{-5})
1000
100
10
1
*
JP26
uvrD::aphA
HPuD comp
ECuD comp
recA::aphA
H. pylori strain designation

B.


Phenotype
Kan
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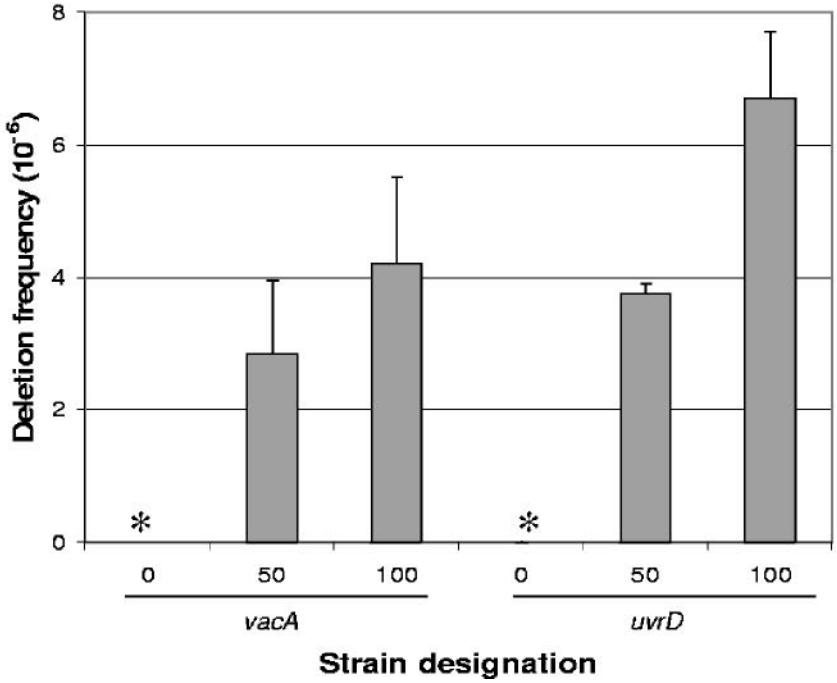
Deletion frequency (10^{-6})
8
6
4
2
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vacA
uvrD
Strain designation

1,400 kJ/m^2 exposures). That the *H. pylori* UvrD homolog could complement the UV sensitivity of *E. coli uvrD* mutants indicated that HP1478 encodes a UvrD homolog and that this protein can interact with other NER proteins in *E. coli* (Fig. 3A).

Since *H. pylori* possesses homologs to all four NER proteins (UvrABCD) (5, 60) and since a role of *H. pylori* UvrB in UV repair has been shown previously (59), to understand UvrD NER function in an *H. pylori* background we next performed UV susceptibility assays using wild-type *H. pylori* strain JP26 and JP26 *uvrD*::*aphA*, an isogenic mutant (Fig. 3B). JP26 *recA*::*aphA* was used as a control, since *recA* mutants are highly susceptible to all types of DNA damage involving recombinational repair (39). As expected, the *recA* mutant showed extreme sensitivity to UV exposure (54, 58), and there was marked (>5 $\log_{10}$) reduction in survival of JP26 *uvrD*::*aphA* with 1,400 kJ/m^2 UV exposure (Fig. 3B). Complementation of JP26 *uvrD*::*aphA* by expression of *uvrD* (HP1478) downstream of a strong (*ureA*) promoter restored the phenotype to nearly wild-type levels (Fig. 3B), demonstrating that the UV susceptibility phenotype was specific to the *uvrD* mutation and not due to polar effects on downstream genes. The *H. pylori uvrD* mutant also was complemented in *trans* through the expression of *E. coli uvrD* in the same *ureA* locus (Fig. 3B). There was partial (>2 $\log_{10}$) yet significant ($P < 0.05$) complementation by *E. coli uvrD*. This result obtained using *H. pylori* cells contrasts with the findings in the *E. coli* background, where the heterologous homolog was functionally equivalent to the native product (Fig. 3A). Thus, more-stringent *H. pylori*-specific requirements for UvrD structure and NER function exist in *H. pylori* than in *E. coli.*

Intergenomic recombination in mutant and complemented *H. pylori* strains. In *E. coli*, *uvrD* mutants are hyperrecombinant (8, 18, 38, 67), but numerous factors, including disruption of MMR, may contribute to this phenotype. We studied *H. pylori uvrD* mutants, since *H. pylori* lacks MMR. Recombination frequency assays of *H. pylori* wild-type and *uvrD* mutants (Fig. 4A) were conducted by transformation of these cells with a linear 800 bp *rpsL* PCR product that confers streptomycin resistance upon recombination (29). For the control cells (JP26 *recA*::*aphA*), which are unable to undergo recombination, no transformants were detected, as expected. JP26 *uvrD*::*aphA* showed a significant (66-fold) increase in intergenomic recombination frequencies compared to wild-type results ($P < 0.05$). As observed for UV susceptibility, in *trans* complementation with *H. pylori uvrD* essentially completely restored the wild-type phenotype, whereas complementation with the *E. coli uvrD* only partially restored the wild-type phenotype ($P < 0.05$ compared with *uvrD*::*aphA*). Thus, *H. pylori* UvrD limits recombination, possibly in conjunction with other NER proteins such as UvrB, with which it physically interacts in *E. coli* (2), or independently of the NER pathway, possibly through repair of stalled replication forks (19), preventing genome rearrangements.

To distinguish between these possibilities, we also examined the recombination frequencies of *H. pylori* JP26 *uvrB* and *uvrC* mutants, which are defective in NER. In contrast to the *uvrD* mutant, the *uvrB* and *uvrC* mutants do not show recombination frequencies significantly different from those seen with wild-type *H. pylori* (see Fig. S2 in the supplemental material), indicating that defective NER will not necessarily lead to hyperrecombination. In total, our results indicate that the increased recombination in the *H. pylori uvrD* mutant ($P < 0.05$) results from independent UvrD helicase activity and does not require interaction with MMR and NER proteins. In *E. coli*, the UvrD helicase dismantles RecA-mediated strand invasion events (63), and it is possible that UvrD has a similar role in *H. pylori*, thereby limiting genetic diversification.

Effect of *uvrD* on deletions between flanking DNA repeats. Since prokaryotic NER mutants have increased numbers of deletions between tandem repeats (11, 21), we next examined the role of *H. pylori* UvrD in deletions involving such direct DNA repeats. We employed a constructed chloramphenicol resistance cassette with flanking identical repeats of 50 or 100 bp (7, 31) inserted into *uvrD* (to produce a null mutation) or into *vacA* as a control (no known effect of its product on recombination) (7). There were small but not significant ($P > 0.05$) increases in deletion frequency for the *uvrD* mutants possessing the 50 and 100 bp identical repeats compared to the results seen with the wild-type (*vacA*) mutants with the parallel constructions (Fig. 4C); thus, UvrD did not significantly influence deletion frequencies under the basal growth conditions.

DNA damage-induced recombination in wild-type strains and *uvrD* mutants. In NER-deficient eukaryotes, UV-induced damage increases chromosomal rearrangements, translocations, and recombination (3, 4, 21, 62). The role of NER in preventing recombination events in prokaryotes has not been

FIG. 4. Inter- and intragenomic recombination in *H. pylori* wild-type and mutant strains. (A) Recombination frequencies. *H. pylori* strains were transformed to streptomycin resistance with an 800 bp A128G *rpsL* PCR product; bars represent means ± standard deviations of recombination frequency. The strains transformed were the JP26 wild type and its *recA* and *uvrD* mutants and the *uvrD* mutant complemented with either HP1478 or *E. coli uvrD* downstream of the *ureA* promoter to create JP26 HPuDcomp or ECuDcomp, respectively. The asterisk indicates that no recombinants were observed for the *recA* strain, as expected. The *uvrD* mutant showed a significant ($P < 0.05$) increase in recombination frequency compared to wild-type results. Both *H. pylori* (HPuDcomp) *uvrD* and *E. coli* (ECuDcomp) *uvrD* complement the *uvrD* mutant to a significant ($P < 0.05$) degree, with *H. pylori uvrD* complementing to a greater extent than *E. coli uvrD.* (B) Schematic of constructs used to assess deletion frequency in *H. pylori. H. pylori* mutants were created by inserting a deletion cassette construct with either 0 bp (control) or 100 bp flanking identical repeat segments (IDS) within *vacA* (control) or HP1478 (indicated as KO gene 5′ and KO gene 3′) (7). Insertion of the complete cassette into a host *H. pylori* strain confers resistance to chloramphenicol. The chloramphenicol cassette can subsequently be deleted by recombination between the two flanking IDS DNA repeats, resulting in resistance (R) to kanamycin and susceptibility (S) to chloramphenicol. (C) Deletion frequencies. The deletion cassette, as described above, was inserted into *vacA* (control) or HP1478, the insertion was confirmed, and deletion frequencies were calculated. Asterisks indicate that no deletions were detected (frequency $< 10^{-8}$). As expected, *H. pylori* strains with the cassette in *vacA* or HP1478 showed progressively higher deletion frequencies with increasing IDS size. Strains with the deletion cassette in HP1478 show a small but not significant ($P = 0.06$) difference in deletion frequencies between flanking DNA repeats of 50 and 100 bp compared to control (*vacA*) mutants with comparable cassettes. Bars represent means ± standard deviations for four to six replicate experiments.

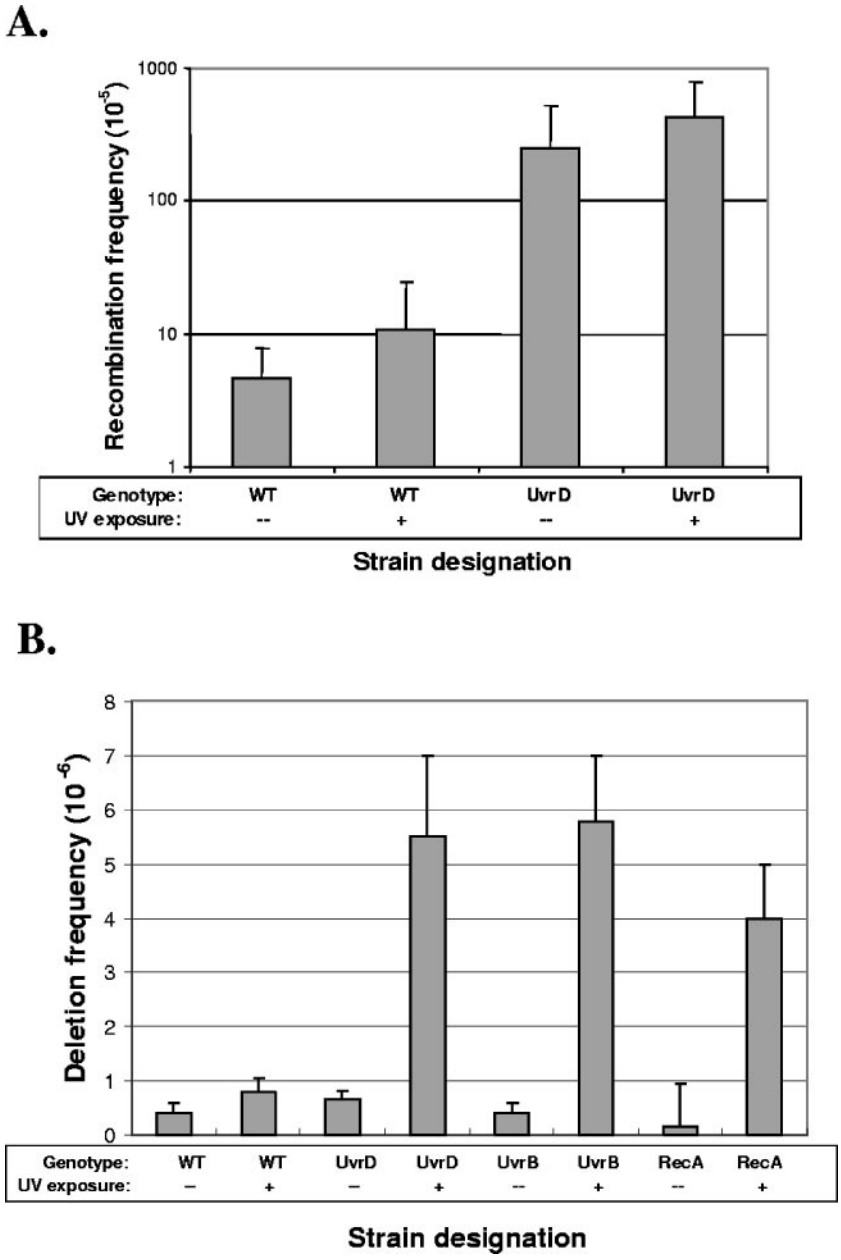

FIG. 5. Influence of DNA damage on *H. pylori* genome rearrangements. (A) Influence of UV exposure on intergenomic recombination. Recombination frequencies were measured using the 800 bp *rpsL* PCR product for *H. pylori* strains JP26 and JP26 *uvrD*::*aphA*, with 0 (control) or 700 kJ/m^2 exposure to UV. As expected, with no UV exposure, the intergenomic recombination frequency of JP26 *uvrD*::*aphA* was higher (62-fold) and significantly ($P < 0.05$) different from the frequency seen with wild-type JP26. With 700 kJ/m^2 of UV exposure, the intergenomic recombination frequencies for both wild-type JP26 and JP26/*uvrD*::*aphA* did not change significantly ($P > 0.05$) from the results for the unexposed control. (B) Influence of UV exposure on deletion frequency. Deletion frequencies were measured as indicated for *H. pylori* strains JP26 *vacA*::*100*, *uvrD*::*100*, *uvrB*::*100*, and *recA*::*100* with no (control) or 700 kJ/m^2 exposure to UV. With no UV exposure, there was no significant difference between the *vacA*::*100* and *uvrD*::*100* deletion strain results, as expected, or between *uvrB*::*100* or *recA*::*100* results. After 700 kJ/m^2 of UV exposure, JP26 *vacA*::*100* displayed a twofold increase in deletion frequency ($P < 0.05$) compared to the results seen with unexposed JP26 *vacA*::*100*. With the UV exposure, the JP26 *uvrD*::*100* strain displayed a 9-fold increase ($P < 0.05$) in deletion frequency compared to unexposed JP26 *uvrD*::*100*, the JP26 *uvrB*::*100* strain displayed a 14-fold increase ($P < 0.05$) in deletion frequency compared to unexposed JP26 *uvrB*::*100*, and the JP26 *recA*::*100* strain showed a 27-fold increase ($P < 0.05$) in deletion frequency compared to unexposed JP26 *recA*::*100*.

characterized as completely, although UvrA and UvrB can suppress certain UV-induced recombination events in *E. coli* (24). Thus, we explored the role of *H. pylori* UvrD in DNA damage-induced rearrangements. First, wild-type and *uvrD* mutant *H. pylori* strains were exposed to UV (312 nm); by use of the 800 bp transforming DNA fragment (Fig. 4A), intergenomic recombination frequencies were assayed. The marginal increases in recombination frequency in both the wild-type and *uvrD* strains after UV exposure (Fig. 5A) indicated that UV-induced DNA damage has little effect on the already

high rates of intergenomic recombination in *H. pylori* and that the DNA damage created by UV exposure does not lead to an increased propensity for recombination.

We also examined the role of UvrD in DNA damage-induced intragenomic recombination, using the constructed *H. pylori* strains in which the deletion cassette was inserted into *uvrD*, *uvrB*, *recA*, or *vacA* (as a control). In the absence of UV, the wild type and the *uvrD* mutant had similarly low deletion frequencies (Fig. 5B), confirming our prior findings (Fig. 4C). Results were parallel for *uvrB* and *recA*, as anticipated by prior results (7). However, after UV exposure, the $\log_{10}$ increase of approximately 1 in deletion frequency in the *uvrD* mutant was significantly ($P < 0.05$) greater than in the wild-type background (Fig. 5B). The *recA* mutant also displayed an increase in deletion frequencies, consistent with the fact that intragenomic recombination between homologous sequences can be RecA independent (37). Parallel studies were undertaken of the *uvrB* mutant, which acts upstream of *uvrD*, to determine whether other components of the NER pathway would display a similar phenotype. In both the absence and presence of UV exposure, results for the *uvrB* and *uvrD* mutants were nearly identical, providing evidence that DNA damage induces intragenomic recombination in *H. pylori*, especially in NER-deficient strains.

DISCUSSION

Does HP1478 encode a UvrD protein? The UV sensitivity of the HP1478 mutant, the partial cross-complementation by *E. coli* UvrD, and the partial complementation of the *E. coli uvrD* mutant by HP1478 provide phylogenetic and functional evidence that HP1478 is indeed a UvrD homolog. The partial complementation for both *E. coli* and *H. pylori* likely reflects primary and secondary structure differences between the two species, which may limit proper interaction between UvrD and other proteins of the NER pathway. That *H. pylori* UvrD can complement *E. coli* in the NER but not the MMR pathway suggests UvrD functional divergence from its *E. coli* counterpart consistent with the absence of *H. pylori* MMR. The closely related *Wolinella succinogenes*, *Campylobacter*, and *Helicobacter* species all lack MMR pathway homologs (16, 30), suggesting that these organisms may have lost MMR function during evolution. Interestingly, homologs of UvrD remain present in these species, suggesting that our findings for *H. pylori* may be applicable to other MMR-deficient organisms as well and that UvrD function, possibly due to its involvement in NER as well as its independent functions, has been retained.

As with other species, *H. pylori uvrD* mutants display sensitivity to UV exposure and increased frequency of intergenomic recombination (33, 47, 48). Increased sensitivity to UV likely results from inability of the cell to remove excised damaged DNA strands, one of the final steps in the NER pathway (Fig. 1), in the absence of a functional UvrD helicase. Increased recombination in *uvrD* mutants can arise through several possible mechanisms—absence of the appropriate helicase may create a stall in the NER pathways (or in MMR in organisms with intact pathways), with resultant generation of loose, recombinogenic DNA strands. It is also possible that UvrD acts independently as a helicase at fork-like DNA substrates, to unwind invading strands; as a 3′→5′ DNA helicase (26, 27, 53), UvrD may limit recombination by unwinding strand invasion events (D loops) and aborting potential recombination events (Fig. 1), consistent with UvrD disruption of RecA-mediated strand invasion in *E. coli* (44). Our experiments show that for *H. pylori*, the latter possibility most likely causes the increased recombination frequencies in *uvrD* mutants, since *H. pylori* lacks MMR. That exposure to UV, which increases substrates for NER, did not lead to increased recombination in *uvrD* mutants compared to wild-type results suggests that a stall in NER is not necessarily responsible for hyperrecombination in *uvrD* mutants and again points to an independent UvrD activity in limiting *H. pylori* genome rearrangements. That *uvrB* and *uvrC* mutants do not display increased recombination is consistent with this hypothesis (see Fig. S2 in the supplemental material).

In contrast, under basal conditions for intragenomic recombination, the UvrD mutant and the wild type showed similar phenotypes, but after DNA damage, there was significant elevation in intragenomic recombination frequencies of the *uvrD* mutants only. These findings indicate a different mechanism for generation of deletions between direct repeats compared to intergenomic recombination, which is consistent with deletions occurring in a RecA-independent process (7), whereas intergenomic recombination requires RecA function (54, 58). We propose the following explanations for these observations. First, the identical repeats are within 1.3 kb of one another (7); processes leading to strand breakage increase chances of slipped-strand mispairing and subsequent deletion of genetic material. DNA damage, especially in NER-deficient strains, results in increased strand breakage potential, potentially leading to increased deletions. Consistent with this explanation, the *H. pylori uvrB* mutant, which is as sensitive to UV damage as the *uvrD* strain (59), shows a DNA damage-induced deletion phenotype similar to that seen with the *uvrD* strains. A second possibility is that in the absence of adequate NER of UV-induced lesions, an alternative pathway for repair, such as recombinational repair, becomes predominant. RecA-mediated recombination also can lead to deletions between identical repeats in *E. coli* (36), and it is possible that when repair pathways become saturated due to defective NER, recombinational repair increases deletion rates. However, recombinational repair requires RecA function; that RecA mutants also exhibit increased deletion frequencies upon DNA damage (Fig. 5B) suggests that strand breakage and subsequent slipped-strand deletions may be a possible mechanism behind DNA damage-induced deletions in *uvrD*, *uvrB*, and *recA* mutants.

Thus, *H. pylori* UvrD has a major influence in limiting intergenomic recombination and deletions, particularly in response to genomic damage. Our studies suggest that this role of UvrD in maintaining genomic fidelity might be critical in vivo, since *H. pylori* lives in an environment that generates substantial genotoxic stress (9, 13, 46).

H. pylori has a high number of repetitive DNA elements that cluster around loci that can potentially influence immunogenicity and host response (7), suggesting that deletion and expansion may be adaptive strategies to target variation to specific locations; in specific contexts, particular phenotypes may have a selective advantage. Our results indicate that DNA damage accelerates the generation of variants in such loci. Thus, UvrD, along with other NER pathway products (e.g.,

UvrB), is crucial in modulating the rate of *H. pylori* variation by limiting DNA damage-induced rearrangements. The unexpected relatively high homology of UvrD from *H. pylori* with those of the extremophiles *T. maritima* and *A. aeolicus*, which have specialized DNA repair systems (40), may reflect that all of these organisms share environments characterized by high levels of genotoxic stress. The genome of *T. maritima* also possesses a high density of repetitive elements, with evidence of frequent lateral transfer and of genetic insertions and deletions (43), in similarity to that of *H. pylori* (7, 17, 56). These similarities suggest that inter- and intragenomic recombination provides a mechanism for adaptation of residents in such extreme environments, and we speculate that UvrD in these organisms has convergently evolved to limit these processes.

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