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Effect of Host Species on RecG Phenotypes in *Helicobacter pylori* and *Escherichia coli*

Josephine Kang,* Don Tavakoli, Ariane Tschumi, Rahul A. Aras, and Martin J. Blaser

Departments of Microbiology and Medicine, New York University School of Medicine, and VA Medical Center, New York, New York

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Recombination is a fundamental mechanism for the generation of genetic variation. *Helicobacter pylori* strains have different frequencies of intragenomic recombination, arising from deletions and duplications between DNA repeat sequences, as well as intergenomic recombination, facilitated by their natural competence. We identified a gene, hp1523, that influences recombination frequencies in this highly diverse bacterium and demonstrate its importance in maintaining genomic integrity by limiting recombination events. HP1523 shows homology to RecG, an ATP-dependent helicase that in *Escherichia coli* allows repair of damaged replication forks to proceed without recourse to potentially mutagenic recombination. Cross-species studies done show that hp1523 can complement *E. coli recG* mutants in *trans* to the same extent as *E. coli recG* can, indicating that hp1523 has *recG* function. The *E. coli recG* gene only partially complements the hp1523 mutation in *H. pylori*. Unlike other *recG* homologs, hp1523 is not involved in DNA repair in *H. pylori*, although it has the ability to repair DNA when expressed in *E. coli*. Therefore, host context appears critical in defining the function of *recG*. The fact that in *E. coli recG* phenotypes are not constant in other species indicates the diverse roles for conserved recombination genes in prokaryotic evolution.

Genetic recombination is an important driving force behind evolution of microbial pathogens, generating new genotypes more rapidly than is possible by mutation alone (11, 22). The acquisition of virulence factors, antigenic determinants, and antibiotic resistance is facilitated by recombination, which creates phenotypically diverse variants while alleviating the cost of high mutation rates by purging deleterious mutations (39, 47).

Helicobacter pylori is a gram-negative, microaerophilic gastric bacterium that persistently colonizes a large proportion of the world's human population (50). The panmictic population structure of *H. pylori* is believed to result from frequent recombination during mixed colonization by unrelated strains (2, 18, 59). Computational analysis of the fully sequenced *H. pylori* strains 26695 and J99 also has identified a large number of direct DNA repeats (1, 6, 54). Intragenomic recombination between such repeats allows deletion or duplication of intervening DNA segments, generating novel subtypes with changes in virulence effectors, such as CagA and CagY, and alterations in restriction modification systems (5, 7, 8).

Although the role of recombination in the generation of *H. pylori* variants with differential pathogenicity is well documented (6, 29, 51, 55, 58), knowledge of the molecular mechanisms that mediate genetic exchange remain rudimentary. Much research about bacterial recombination has focused on *Escherichia coli*; several enzymes that suppress recombination have been identified, including the RecG and RecQ helicases (23, 44), and the mismatch repair proteins MutS, MutH, and MutL (19, 53). Although *H. pylori* lacks homologs of MutS, MutH, and MutL, a putative RecG homolog, HP1523, has been identified by in silico analysis of sequenced *H. pylori* strain 26695 (JHP1412 in strain J99). Helicases, such as RecG, limit genome rearrangement by acting at damaged replication forks to repair lesions without recourse to recombination (44), a function possibly important in maintaining the genomic integrity of highly recombining species, such as *H. pylori*.

Therefore, we studied HP1523 to determine whether it has RecG functions and whether these functions influenced inter- and intragenomic recombination. Cross-species complementation studies performed between *E. coli* and *H. pylori* suggest that their RecG proteins are functionally interchangeable in *E. coli* but display phenotypic differences reflecting divergent intracellular environments. Unlike other RecG homologs (34, 41, 48), the *H. pylori* RecG (encoded by hp1523), is not required for recovery from DNA damage, although it plays a role in limiting recombination. These findings suggest that HP1523, influencing genomic plasticity in *H. pylori* without involvement in DNA repair pathways, functions in a new role for RecG helicase homologs.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* and *H. pylori* strains and plasmids used in this study are listed in Table 1. *E. coli* strains 1157 (9) and N4452, kindly provided by Robert Lloyd, were routinely grown in Luria-Bertani broth (LB) at 37°C. *H. pylori* strains were routinely grown on Trypticase soy agar (TSA) plates at 37°C in a 5% CO_2 incubator.

Construction of *H. pylori* mutants used to assess susceptibility to UV, intergenomic recombination frequencies, and spontaneous mutation frequencies. Fragments of HP1523 (*recG* homolog), HP0887 (*vacA*), and HP0153 (*recA*) open reading frames (ORFs) were amplified by PCR using primers based on sequenced strain *H. pylori* 26695 or J99 (Table 2) and cloned into pGEMT-Easy (Promega, Madison, Wis.) to create p1523, pVacA, and pRecA, respectively. Next, p1523 was cut with EcoRI and ligated to an *aphA* cassette from pUC4K (GenBank accession no. X06404), which confers kanamycin resistance (Km^r), to create p1523Km. Inverse PCR was performed on pVacA and pRecA to introduce BamHI sites (see Table 2 for primers), and the PCR products were digested and subsequently ligated to *aphA* to create 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. The *recA* locus was interrupted

* Corresponding author. Mailing address: Department of Medicine, New York University School of Medicine, 550 First Ave., New York, NY 10016. Phone: (212) 263-4105. Fax: (212) 263-7700. E-mail: kangm01@med.nyu.edu.

TABLE 1. Plasmids and bacterial strains used in this study

Plasmid or strain	Relevant characteristic(s)	Source or reference
Plasmids		
p1523	HP1523 fragment (bp 343–1520) in pGEMT-Easy	This work
pVacA	VacA fragment (bp 798–2003) in pGEMT-Easy	This work
pRecA	RecA fragment (bp 1–1040) in pGEMT-Easy	This work
pRecGKm	HP1523::*aphA* in pGEMT-Easy	This work
pVacAKm	*vacA*::*aphA* in pGEMT-Easy	This work
pRecAKm	*recA*::*aphA* in pGEMT-Easy	This work
pVacA-0	*vacA* with 0-bp deletion cassette	6
pVacA-50	*vacA* with 50-bp deletion cassette	6
pVacA-100	*vacA* with 100-bp deletion cassette	6
pRecG-0	HP1523 with 0-bp deletion cassette	This work
pRecG-50	HP1523 with 50-bp deletion cassette	This work
pRecG-100	HP1523 with 100-bp deletion cassette	This work
pAD1	*ureA* promoter	4
pAD1-HPrG	pAD1 with HP1523	This work
pAD1-ECrG	pAD1 with *E. coli recG*	This work
Strains		
E. coli		
AB1157	*recG*+ (wild type)	11
N4452	AB1157 *recG*::*cat*	29
N4452-AD1	N4452 with pAD1 (control)	This work
N4452-HPrG	N4452 complemented with HP1523	This work
N4452-ECrG	N4452 complemented with *E. coli recG*	This work
H. pylori		
JP26	Wild-type strain	
JP26/HP1523::*aphA*	ΔHP1523	This work
JP26/*vacA*::*aphA*	ΔVacA	This work
JP26/*recA*::*aphA*	ΔRecA	This work
JP26 HPrG	HP1523::*aphA* complemented with HP1523	This work
JP26 ECrG	HP1523::*aphA* complemented with *E. coli recG*	This work
JP26/HP1523:0	HP1523 interrupted with 0-bp deletion cassette	This work
JP26/HP1523:50	HP1523 interrupted with 50-bp deletion cassette	This work
JP26/HP1523:100	HP1523 interrupted with 100-bp deletion cassette	This work
JP26/*vacA*::*0*	*vacA* interrupted with 0-bp deletion cassette	6
JP26/*vacA*::*50*	*vacA* interrupted with 50-bp deletion cassette	6
JP26/*vacA*::*100*	*vacA* interrupted with 100-bp deletion cassette	6

as another control, since several phenotypes related to this study have been characterized (6, 62). By natural transformation as described previously (67), *H. pylori* strain JP26 was transformed to Km^r with p1523Km, pVacAKm, and pRecAKm to create JP26 1523::*aphA*, *vacA*::*aphA*, and *recA*::*aphA*. Chromosomal DNA was isolated from mutant strains, 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 intragenomic (deletion) frequencies. A unique BamHI site was created in p1523 and pVacA by inverse PCR using primers based on sequenced strain 26695 (Table 2), and each ORF was subsequently interrupted with a deletion cassette containing either 0-, 50-, or 100-bp repeats to create p1523-0, -50, and -100 and pVacA-0, -50, and -100. The construction and use of the deletion cassettes have been documented previously (6). *H. pylori* strain JP26 was subsequently transformed to chloramphenicol resistance (Cat^r) with these plasmids by natural transformation to create JP26 HP1523::0, -50, and -100 and JP26 *vacA*::0, -50, and -100. Chromosomal DNA was isolated from mutant strains, and the correct insertion of the deletion cassette into the expected ORF was confirmed by PCR in each case.

Deletion frequency assays in *H. pylori* to assess intragenomic recombination. 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

TABLE 2. Primers used in this study

Primer	Gene	Location[a]	Sequence (5′ → 3′)[b]
F343	HP1523	1553772–1553794	ATCCTTACCGAGTTTGGCAAGA
R1520	HP1523	1552617–1552637	GAAATGCCCACCTCAATGAG
R483	HP0153 (*recA*)	163411–163430	TTGGATCCGCATGTGGGCTTGCAAGCA
F477	HP0153 (*recA*)	163385–163405	CTTGGATCCCCATCAATCTCCGCTTTAGG
F1	HP0153 (*recA*)	162928–163405	ATGGCAATAGATGAAGACAAAC
R1040	HP0153 (*recA*)	163944–163968	TCCATTTCTTCTAAAGGCTCATCC
R1060	HP0887 (*vacA*)	939456–939475	TACGGGATCCTTTTCCTGCTTGAATGCGC
F1082	HP0887 (*vacA*)	939497–939517	AAGAGGATCCAAGGAATACGACTTATACA
F798	HP0887 (*vacA*)	939213–939231	CGTGAAAGCGAAAAACAAG
R2003	HP0887 (*vacA*)	940400–940418	CATCGGCTTTAGTGTTGA

[a] HP1523 locations based on the sequence of strain J99. HP0153 (*recA*) and HP0887 (*vacA*) locations based on the sequence of strain 26695.
[b] BamHI restriction sites underlined.

at 37°C (5% CO_2), allowing deletions to occur. The cells were harvested and washed twice in phosphate-buffered saline (PBS), and 25-, 100-, and 200-μl aliquots were spread on brucella broth agar (BA) plates supplemented with newborn calf serum (NCS) and 25 μg of kanamycin per ml. For additional controls, 200 μl from each suspension was inoculated onto 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 (6). Total CFU and numbers of Km^r deletion mutants were determined by plating serial dilutions onto TSA plates alone or TSA plates with kanamycin (25 μg/ml), respectively. Plates were incubated at 37°C in a 5% CO_2 environment for 96 h, colonies were counted, and deletion frequencies were calculated.

Streptomycin resistance frequency assay to assess intergenomic recombination. *H. pylori* strains were grown on TSA plates with 5% sheep blood agar. After 48 h, the recipient *H. pylori* cells were harvested and placed in 1 ml of PBS, and 25 μl of the resulting suspension was combined with 30 ng of donor DNA by spotting onto a TSA plate. The TSA plate was 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. The transformation mixture was then harvested and placed in 1 ml of PBS, and 100-μl portions of the appropriate serial dilutions were plated onto either TSA plates or BA plates containing 10% NCS and 25 μg of streptomycin per μl. The plates were incubated for 4 days at 37°C in 5% CO_2, and the total recombination frequency was determined by the number of St^r colonies divided by the total CFU. For a negative control, *H. pylori* strains with no DNA added were also tested in parallel with each experiment; no colonies were seen in any case.

Assays to examine recovery from DNA damage. *H. pylori* cells to be tested were grown on TSA plates for 48 h and suspended in 1 ml of brucella broth. Equal amounts of suspension were inoculated on TSA plates to produce 100 to 500 CFU per plate. Cells were exposed to UV radiation at a wavelength of 312 nm (Stratagene Transilluminator) for 0 to 60 s and incubated at 37°C in 5% CO_2 for 96 h. Colonies were counted, and the percentage of survival was calculated.

Ciprofloxacin E-test strips (AB Biodisk, Solna, Sweden) were used to determine the MICs for both wild-type and mutant strains of *H. pylori* and *E. coli*, according to the manufacturer's instructions. Plates were incubated for 48 and 24 h, respectively, and MIC determinations were repeated at least three times for each sample.

Morphology of *H. pylori* mutant cells. Cells of wild-type and mutant *H. pylori* and *E. coli* strains, grown to stationary phase at 48 and 24 h, respectively, were spread onto glass slides and Gram stained. After examining the cells with a light microscope, the percentages of *H. pylori* and *E. coli* cells showing filamentation were determined.

Complementation of the *H. pylori* JP26 *recG*::*aphA* mutant. Plasmid pAD1-HPrG, with ORF HP1523 placed downstream of the *ureAB* promoter, was constructed by using the same methods as those used to make pANDO2 (4), except HP1523 replaces HP0333. pAD1-HPrG was used to introduce HP1523 in *trans* into the genome of mutant JP26 *recG*::*aphA* via natural transformation as described previously (4, 67) to create JP26 HPrG comp. Transformants were selected on the basis of Cat^r, and the correct insertion of *recG* and flanking regions into *ureA* was confirmed by PCR of the chromosomal DNA. In parallel experiments, pAD1-ECrG (see below) was used to construct JP26 ECrG comp, exactly as described above.

Complementation of *E. coli recG* mutant. pAD1-ECrG was constructed by inserting the *E. coli recG* ORF downstream of the *ureA* promoter, as described above, based on the construction of pANDO2 (4). *E. coli* strain N4452 (*recG* null mutant) was transformed with either pAD1-HPrG, pAD1-ECrG, or pAD1 (no insert) to create strains N4452-HPrG, N4452-ECrG, and N4452-AD1, respectively. For *E. coli* strains 1157 (wild type), N4452, N4452-HPrG, N4452-ECrG, and N4452-AD1, recovery after exposure to DNA damage was assessed using cultures that were grown overnight and serially diluted onto TSA plates and exposed to 312-nm-wavelength UV light (Stratagene Transilluminator) for 0 to 80 s. After incubation of plates overnight at 37°C, total numbers of colonies were counted, and survival was determined.

Statistical analyses. Student's *t* test, unpaired with equal variance, was used to determine statistical significance in all cases. A P value of <0.05 was defined as statistically significant.

RESULTS

Comparison of HP1523 with RecG helicases. Previous analyses have shown that RecG is highly conserved in a wide range of bacteria and in plants (37, 56). A RecG homolog, HP1523, was identified in *H. pylori* sequenced strain 26695 using the clusters of orthologous groups of proteins (COG) database (http://www.ncbi.nlm.nih.gov/COG) (61), with 98.7% similarity to the product of JHP1412 in *H. pylori* strain J99 and 53.6% similarity to *E. coli* RecG. We first sought to determine the extent of similarity between HP1523 and members of the RecG family. The amino acid sequences of HP1523 and JHP1412 were aligned with RecG sequences from several representative bacterial species (Fig. 1A). The N terminus of RecG recognizes branched DNA structures and displays less similarity among the different bacterial species than do other parts of the protein (38, 56); the *H. pylori* products are no exception. The C-terminal region contains the helicase active site (38, 56), and the highly conserved RecG helicase motifs (35) are present in HP1523 and JHP1412. These studies support the annotation of HP1523 and JHP1412 as encoding RecG homologs (3, 64).

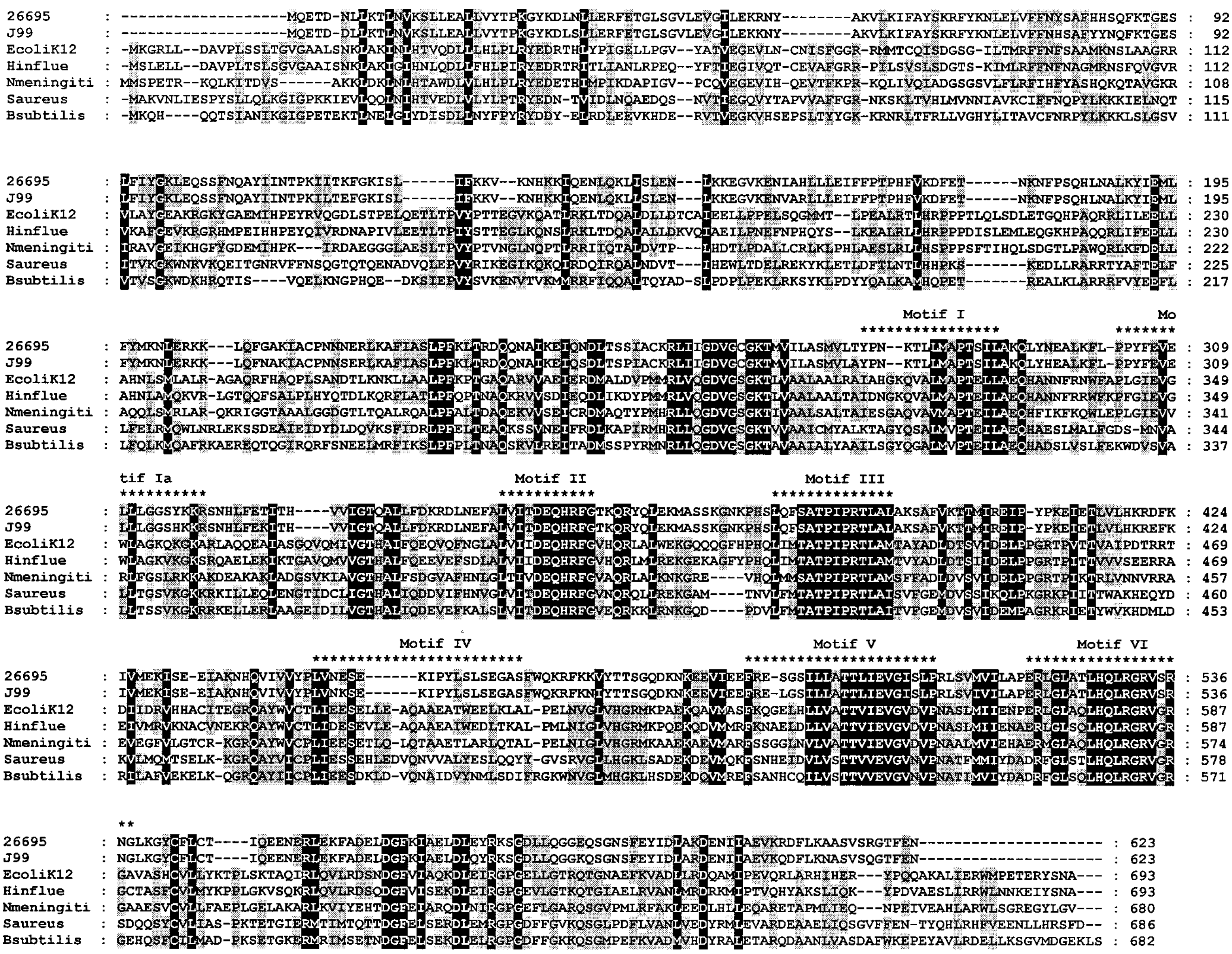
Phylogenetic analysis of the deduced HP1523 and JHP1412 products with RecG from several representative bacterial species (Fig. 1B) shows that the closest relationship is with *Campylobacter jejuni*, as expected. However, unexpectedly, the *H. pylori* and *C. jejuni* orthologs are most closely related to those in gram-positive bacteria, rather than other gram-negative bacteria.

Effect of HP1523 on recombination frequency. In *E. coli*, RecG influences recombination by converting halted replication forks into Holliday junctions and facilitating direct repair of such lesions (44). As a helicase that recognizes a variety of branched DNA structures, RecG also acts at D-loops, which are recombination intermediates formed during strand invasion of double-stranded DNA (43). By facilitating the direct repair of replication fork lesions without the need for recombination and through the dissolution of recombination intermediates, RecG helicase limits recombination (24, 43, 45).

To determine whether HP1523 has a similar role in limiting recombination, we compared the frequency of natural transformation in wild-type and hp1523 mutant *H. pylori* strains. The hp1523 mutant displayed a >10-fold increase in intergenomic recombination frequencies over the wild type (Fig. 2) and a parallel *aphA* mutant of *vacA*, which has no known recombination activity. As expected, in a *recA* mutant, no transformants were detected. To confirm that the observed phenotype was not due to polar effects on genes downstream of HP1523, the hp1523 mutant was complemented in *trans* through integration of the hp1523 sequence downstream of a strong (*ureA*) promoter at a distant chromosomal locus. Complementation restored the wild-type phenotype (Fig. 2). In total, these results are consistent with a *recG* function for hp1523.

Effect of HP1523 on intragenomic recombination between direct DNA repeats. Several types of DNA damage can lead to DNA strand breakage (14, 32, 46, 65). Generation of loose DNA ends, which can participate in recombinogenic events, are hypothesized to promote deletion and duplication of intervening sequences between direct DNA repeats. Since the RecG helicase acts at damaged replication forks to repair DNA damage, it limits the generation of loose ends that can participate in both inter- and intragenomic recombination events. Analysis of the *H. pylori* genome reveals the existence of numerous direct DNA repeat sequences and the potential for genome rearrangements (1, 6, 54). Since the activity of helicases, such as RecG, that can limit genomic plasticity (44),

A.



B.

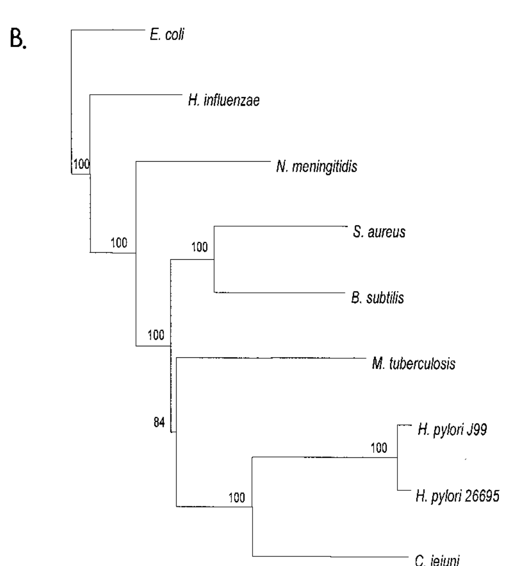


FIG. 1. Relationship of deduced *H. pylori* products with RecG homologs. A. Alignment of amino acid sequences of *H. pylori* HP1523, JHP1412, and RecG homologs from five bacterial species (*E. coli* K-12, *Haemophilus influenzae* [Hinflue], *Neisseria meningitidis* [Nmeningiti], *S. aureus*, and *Bacillus subtilis*). Protein sequences were aligned with ClustalW, and the locations of conserved RecG helicase motifs (35) are indicated with asterisks. The program Genedoc (www.psc.edu/biomed/genedoc) was used to visualize the alignment in quantify mode, which highlights the one, two, or three most-frequent residues found in each column of the alignment. Conservative substitutions (e.g., I, L, V, M) were treated as if identical. Amino acids conserved in all species are indicated by white letters on black background. Gaps introduced to maximize alignment are indicated by dashes. Black letters on gray shading represent 60% or greater identity. White letters on black shading represent 100% identity. B. Phylogeny of RecG orthologs. Phylogenetic analysis of RecG orthologs was performed on 8 representative bacterial species, with bootstrap values listed next to the branches. *M. tuberculosis*, *Mycobacterium tuberculosis*.

may be especially important in organisms prone to genomic instability, such as *H. pylori*, we examined whether HP1523 could influence intragenomic recombination.

To determine whether HP1523 could influence intragenomic recombination, we used a previously validated deletion

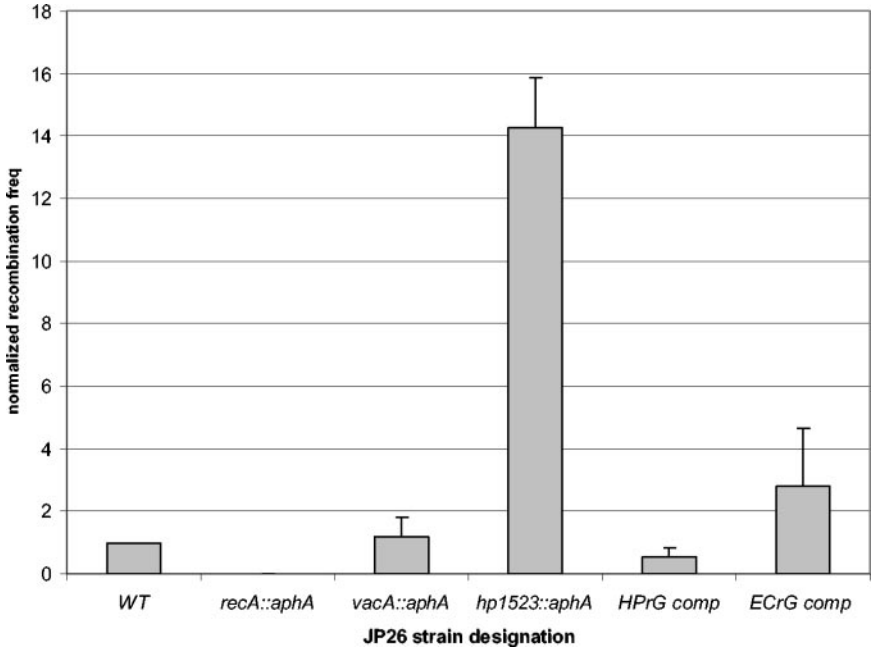


FIG. 2. Intergenomic recombination frequencies (freq) of *H. pylori* wild-type and mutant strains. *H. pylori* strains were transformed to streptomycin resistance with a 800-bp *rpsL* A128G PCR product. The values are the means ± standard deviations (error bars) of transformation frequency normalized to that of the wild-type strain from four to eight replicate experiments. The *H. pylori* strains that were transformed were JP26 (wild type [WT]) and its *recA*, *vacA* (control), and hp1523 mutants, as well as the hp1523 mutant with a copy of either hp1523 or *E. coli recG* in *trans* downstream of the *ureAB* promoter to create JP26 HPrG comp and ECrG comp, respectively. As expected, there was no significant difference in transformation frequencies between the wild-type strain and the *vacA* (control) mutant, and in the *recA* mutant, no transformants were observed. The intergenomic recombination frequency in the *recG* mutant is consistently higher (14-fold) and significantly ($P < 0.05$) different from the transformation frequency of wild-type JP26. Complementation of the hp1523 mutant with hp1523 led to a recombination frequency that was not significantly different from that of the wild type. Complementation of the hp1523 mutant with *E. coli recG* led to a recombination frequency that was lower than the hp1523 mutant but still significantly (*P* value < 0.05) higher than that of the wild type.

cassette (6), with identical DNA repeats (IDS) of 0, 50, or 100 bp (Fig. 3A). The deletion cassette was introduced into hp1523 or *vacA* (as a control) to assess the effect of the hp1523 product on intragenomic recombination between direct repeats. Consistent with the hypothesis that hp1523 is a *recG* homolog, the hp1523 mutant showed a significant (>10-fold) increase in recombination frequency compared to *vacA* mutant control strains for the IDS repeat sizes tested (Fig. 3B). These findings are consistent with hp1523 playing a role in limiting intragenomic recombination between direct DNA repeats.

Recovery from DNA damage. *E. coli recG* mutants are sensitive to DNA-damaging agents, such as UV radiation (34), due to their decreased ability to overcome replication fork-halting lesions. Hypothesizing that the *H. pylori* hp1523 mutant would show a similar survival defect, we exposed mutant and wild-type *H. pylori* strains to UV irradiation, which primarily damages the DNA template by creating pyrimidine dimers (57). However, in contrast to our expectations, the hp1523 mutant did not display a substantial increase in susceptibility to UV damage compared to the wild-type or control strain (Fig. 4).

To examine the role of hp1523 in recovery from a different type of DNA damage, we measured the susceptibility of the hp1523 mutant and wild-type *H. pylori* strains to the fluoroquinolone ciprofloxacin. Fluoroquinolones are bactericidal agents that primarily target DNA gyrase or topoisomerase IV (16, 30), creating a complex composed of protein, fluoroquinolone, and DNA that both halts replication forks and generates loose DNA ends (13, 40). Since *Staphylococcus aureus recG* mutants have increased susceptibility to quinolones, it is believed that RecG helps overcome the damage induced by these ternary complexes by acting at damaged replication forks (48).

To determine whether *E. coli* RecG and HP1523 play similar roles in recovery from quinolone-induced damage, we examined the susceptibilities of *E. coli recG* mutant and wild-type strains and *H. pylori* hp1523 mutant and wild-type strains to ciprofloxacin. Our results indicate that the *E. coli recG* mutant is more susceptible than the wild-type strain to ciprofloxacin, similar to the findings for *S. aureus* (48). In contrast, the hp1523 mutant was slightly less susceptible to ciprofloxacin than wild-type *H. pylori* cells, and complementation of HP1523 in *trans* restores ciprofloxacin resistance to wild-type levels, confirming that the effect is specific to HP1523 (Table 3).

Influence of HP1523 on cell morphology. We then explored bacterial filamentation, another manifestation of DNA damage (25, 31, 42), that has been documented in most bacterial species, including *H. pylori* (15, 28, 36, 60). In *E. coli*, defects in recombination proteins, such as PriA, RecA, and RecG, lead to filamented cells (25, 42, 49). Therefore, using light microscopy, we examined the HP1523 mutant and wild-type *H. pylori* strains and control *E. coli* strains to determine whether filamentation could be observed. As expected, filamentation was apparent (5%) for the *E. coli recG* mutant after 24 h of growth to stationary phase (25), but not in the wild-type strain (<0.1%). For the wild-type *H. pylori* strain, JP26, no (<0.1%) filamen-

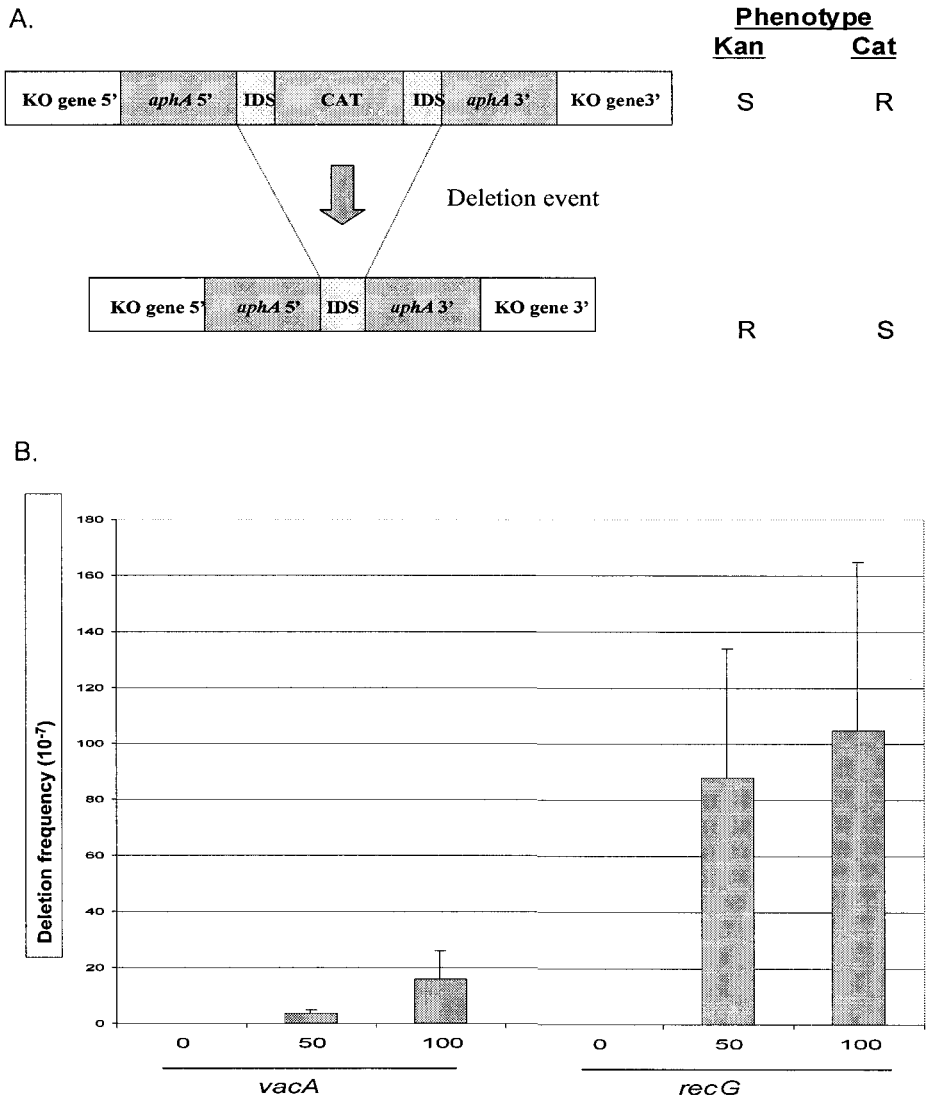


FIG. 3. Intragenomic recombination in *H. pylori* wild-type and mutant strains. A. Schematic of constructs used to assess deletion frequency in *H. pylori*. *H. pylori* mutants were created by inserting a deletion cassette construct with either 0-bp (control), 50-bp, or 100-bp flanking identical repeat segments (IDS) in *recA*, *vacA*, and *recG* (indicated as knockout [KO] gene 5′ and KO gene 3′). One copy of the IDS is part of the 5′ region of *aphA*, and the other has been ligated to the chloramphenicol (CAT) cassette. 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. B. Deletion frequencies. The values are the means ± standard deviations (error bars) from four to six replicate experiments. As expected, *H. pylori* strains with the cassette in *vacA* (control) showed progressively higher deletion frequencies with increasing size of the IDS. Strains with the deletion cassette in *recG* show significantly (25- and 7-fold) higher intragenomic recombination frequencies between flanking DNA repeats of 50 and 100 bp, respectively (P values of <0.05) compared to the control mutants with comparable cassettes in *vacA*.

tation was observed after 48 h of growth (reflecting stationary phase); no filamentation was observed in the HP1523 mutant, in contrast to the *E. coli* findings. Thus, in a second phenotype related to DNA damage, hp1523 appears to differ in function from its *E. coli* homolog.

Cross-species complementation of RecG. Despite the strong similarity of HP1523 to other RecG homologs (Fig. 1), HP1523 does not play a role in survival after DNA damage or filamentation, as is expected for a RecG helicase. These findings suggest either that HP1523 may not be a true member of the RecG family or that the milieu in which recombination and DNA repair proteins operate differs in *E. coli* and *H. pylori*, resulting in different RecG phenotypes. To distinguish between these possibilities, as well as to determine the extent of functional similarity between HP1523 and *E. coli* RecG, cross-species complementation studies were performed between *H. pylori* and *E. coli*.

To determine whether the *E. coli* RecG protein could complement *H. pylori* HP1523 mutants, pAD1-ECrG was used to transform HP1523::*aphA*, creating strain JP26 ECrG comp. Intergenomic recombination frequencies (Fig. 2) and susceptibilities to ciprofloxacin (Table 3) were used to assess pheno-

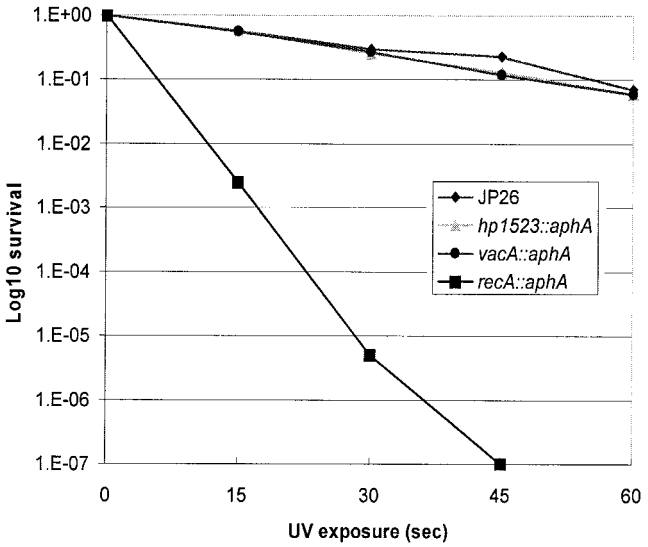


FIG. 4. Susceptibility of *H. pylori* wild-type and mutant strains to UV exposure. *H. pylori* wild-type strain JP26 and *recA*, *recG*, and *vacA* mutant strains were assayed for survival after exposure to UV (wavelength of 312 nm) for 0 to 60 s. As expected, the *recA* mutant showed significantly greater susceptibility to UV compared to that of wild-type JP26. The susceptibilities of *recG* and *vacA* (control) mutants were not significantly different from that of the wild-type strain. The results from one representative experiment are shown (four trials were performed). 1.E-07, 10^{-7}.

type. Our results show that *E. coli* RecG can partially complement *H. pylori* HP1523 mutants, but not to the same extent as native HP1523.

To determine whether the *H. pylori* HP1523 protein could complement in *E. coli*, the *E. coli recG* mutant strain, N4452 (27), was transformed with HP1523 in a shuttle plasmid to create N4452-HPrG, with *E. coli* RecG in the same vector to create N4452-ECrG (positive control), or with the vector alone to create N4452-AD1 (negative control). Susceptibilities to UV exposure and ciprofloxacin were used to assess phenotype. As expected, N4452-ECrG, but not N4452-AD1, displayed an increase in survival after exposure to either UV (Fig. 5) or ciprofloxacin (Table 3), demonstrating that complementation with the *E. coli* gene in *trans* was achieved. After exposure to UV or ciprofloxacin, strain N4452-HPrG also showed survival to nearly wild-type levels (Fig. 5 and Table 3); the findings were nearly identical in magnitude to those achieved in *trans* with *E. coli* RecG. These observations indicate that HP1523 can perform known RecG functions involved in DNA repair when expressed in *E. coli*, but not in *H. pylori*.

DISCUSSION

Recombination is essential for chromosomal repair after DNA damage and also permits generation of variation through intrachromosomal rearrangements and assimilation of foreign DNA (11, 52). Such generation of diversity is important in prokaryotes, facilitating adaptation in response to environmental stresses (1, 54). *E. coli* is widely accepted as a model organism for understanding enzymes involved in recombination, leading to paradigms that have been generalized to other prokaryotes. However, we now show that even among related gram-negative organisms, such as *E. coli* and *H. pylori*, intracellular host milieu is sufficiently different to affect RecG phenotypes. HP1523 (and JHP1412) have been annotated as being RecG homologs (3, 64); our alignment and phylogenetic analyses are consistent with this, and the presence of conserved RecG motifs (35) provides further strong support. Although we conclude that HP1523 is indeed a member of the RecG family, our experimental studies indicate several phenotypic differences.

In previous studies of *S. aureus*, *Streptococcus pneumoniae*, and *E. coli*, RecG has been important in recovery after exposure to DNA-damaging agents (34, 41, 48), whereas our studies indicate that RecG is not involved in DNA repair in *H. pylori* after damage due to exposure to UV (Fig. 4) or methyl methanesulfonate (data not shown). However, the cross-species complementation studies indicate that *H. pylori* RecG has the ability to repair DNA lesions in *E. coli* and complements *E. coli recG* mutants to the same extent as the native *E. coli* RecG. Why, then, is it not involved in *H. pylori* DNA repair?

One possibility is that *H. pylori* has evolved alternative, possibly more efficient mechanisms to repair DNA, obviating the need for RecG in recovery from DNA damage. Genome analysis and prior studies indicate that *H. pylori* has the capacity to repair base and nucleotide excision (63). Having highly competent DNA repair may be especially important for *H. pylori*, which induces host inflammatory responses involving neutrophils, lymphocytes, and macrophages, releasing DNA-damaging free radicals (26).

The repertoire of putative recombination proteins in *H. pylori* differs from that in *E. coli*. RecFO, RecCD, DnaC, and other important recombination pathway components in *E. coli* are not present in *H. pylori* on the basis of in silico analyses in sequenced strains (3, 64), as well as an ortholog search using the COG database (data not shown). Although it is possible that *H. pylori* has alternative recombination pathways that have not been identified yet, the absence of such regulatory enzymes may contribute to its extraordinarily high rate of recombina-

TABLE 3. Susceptibilities of wild-type, *recG* mutant, and complemented *E. coli* and *H. pylori* strains to ciprofloxacin[a]

Strain	Ciprofloxacin MIC (μg/ml)
E. coli	
AB1157	0.018 ± 0.003
N4452	0.008 ± 0.001[b]
N4452-HPrG	0.017 ± 0.002
N4452-ECrG	0.017 ± 0.002
N4452-AD1	0.009 ± 0.001[b]
H. pylori	
JP26	0.103 ± 0.013
JP26/HP1523::*aphA*	0.272 ± 0.040[c]
JP26 HPrG comp	0.106 ± 0.013
JP26 ECrG comp	0.176 ± 0.052[c]

[a] The *E. coli* and *H. pylori* strains were assayed by inhibition within lawns on plates containing a ciprofloxacin E-test strip (AB Biodisk) in concentrations from 0.002 to 32 μg/ml. Results shown are the means (± standard deviations) of at least four replicate determinations.

[b] These values are significantly different ($P < 0.05$) from the value for AB1157 (wild type).

[c] These values are significantly different ($P < 0.05$) from the value for JP26 (wild type).

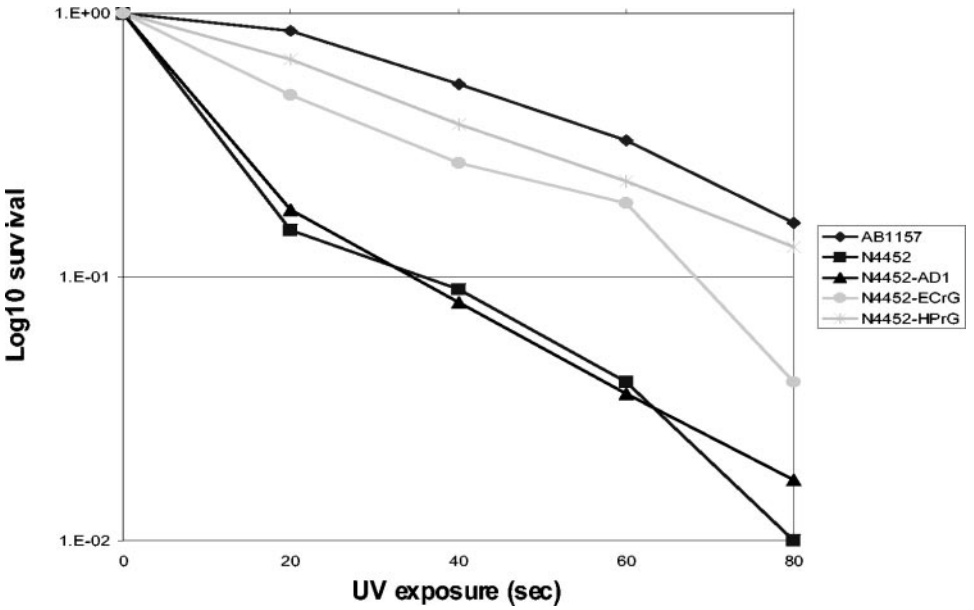


FIG. 5. Susceptibility of cross-complemented *E. coli* strains to UV exposure. *E. coli* strains transformed with a plasmid containing *E. coli* RecG (pAD1-ECrG), *H. pylori* HP1523(pAD1-HPrG), or no insert (pAD1) were examined for susceptibility to UV for 0 to 80 s. The results from one representative experiment are shown (three trials were performed). 1.E-02, 10^{-2}.

tion (59). The only partial complementation by *E. coli* RecG suggests that *H. pylori* RecG has additional domains required for full function in *H. pylori*.

E. coli recG mutants display increased susceptibility to quinolones (Table 3), clearly showing a role for *recG* in repair. The unexpected finding that the *H. pylori recG* mutants are less susceptible to ciprofloxacin than the wild type may result from enhanced recombination, which aids recovery from quinolone-induced DNA damage (16). Thus, the extent of the *recG* mutation on recombination may supersede a more limited effect on repair. The fact that *H. pylori recG* apparently is not involved in repair of UV-induced damage is consistent with this hypothesis, since both UV and quinolones induce replication fork blockage.

Another possibility is that although RecG is not essential for recombinational repair of DNA lesions, it is involved in a redundant repair pathway. The ternary complexes formed by quinolones are not the cause of cell death per se (12, 20, 30, 33, 61), but an additional step, most likely an aborted repair attempt, is required for the generation of a lethal DNA double-strand break (12, 33). Therefore, the repair pathway through which a ternary complex is processed ultimately determines the organism's level of susceptibility. If repair of ternary complexes represents a balance between repair and recombination phenomena, the differing *recG* phenotypes in these respects may explain the observed results. Since *E. coli* and *H. pylori* possess different repair pathway proteins, the presence or absence of RecG may alter quinolone susceptibility in different ways. The cross-species complementation results (Table 3 and Fig. 5) support this hypothesis.

H. pylori colonizes an environmental niche that is essentially isolated from other organisms, unlike *E. coli*, which must compete in its niche with other prokaryotes and is exposed to diverse genetic material. High rates of intragenomic recombination may be a way *H. pylori* can maximize the probability of survival in dynamic host environments (10), allowing self-generation of a genetically diverse population from which the variants that are most fit can be selected (13). In every species, there is an intrinsic tension between fidelity, implied by DNA repair mechanisms, and diversification at particular loci, represented by recombination. In *H. pylori*, as in RNA viruses, the requirement for diversification may be so great that fidelity is a lower priority.

Therefore, the existence of a DNA helicase, RecG, that preserves genomic integrity in this highly diverse organism by limiting both intra- and intergenomic recombination is of interest. Helicases that maintain genome stability by limiting recombination have been found in other bacteria and eukaryotes, including humans (17, 21). The conservation of RecG in *H. pylori*, despite the absence of involvement in DNA repair, suggests that its major role could involve maximizing genomic integrity, especially in *H. pylori*, whose genome contains substantial repetitive DNA that can foster illegitimate exchanges (6).

The increased intergenomic recombination displayed by the *H. pylori recG* mutant is consistent with the prior understanding of RecG function, involving recognition and unwinding of branched DNA structures (43, 66). The fact that the *H. pylori recG* mutants also display increased intragenomic recombination frequencies between direct DNA repeats suggests that the mechanisms involved in deletion include the formation of branched DNA structures. On the basis of studies in *E. coli* (45, 66), we hypothesize that in wild-type *H. pylori* cells, RecG recognizes and unwinds such intermediate structures, preventing many deletion events from reaching completion. Since these studies are the first, to our knowledge, to examine recombination between chromosomal DNA repeats, such a RecG phenotype may be present in other species.

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