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Dual Nuclease and Helicase Activities of *Helicobacter pylori* AddAB Are Required for DNA Repair, Recombination, and Mouse Infectivity*[S]

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Helicobacter pylori infection of the human stomach is associated with disease-causing inflammation that elicits DNA damage in both bacterial and host cells. Bacteria must repair their DNA to persist. The *H. pylori* AddAB helicase-exonuclease is required for DNA repair and efficient stomach colonization. To dissect the role of each activity in DNA repair and infectivity, we altered the AddA and AddB nuclease (NUC) domains and the AddA helicase (HEL) domain by site-directed mutagenesis. Extracts of *Escherichia coli* expressing *H. pylori* $addA^{NUC}B$ or $addAB^{NUC}$ mutants unwound DNA but had approximately half of the exonuclease activity of wild-type AddAB; the $addA^{NUC}B^{NUC}$ double mutant lacked detectable nuclease activity but retained helicase activity. Extracts with $AddA^{HEL}B$ lacked detectable helicase and nuclease activity. *H. pylori* with the single nuclease domain mutations were somewhat less sensitive to the DNA-damaging agent ciprofloxacin than the corresponding deletion mutant, suggesting that residual nuclease activity promotes limited DNA repair. The $addA^{NUC}$ and $addA^{HEL}$ mutants colonized the stomach less efficiently than the wild type; $addB^{NUC}$ showed partial attenuation. *E. coli* Δ*recBCD* expressing *H. pylori addAB* was recombination-deficient unless *H. pylori recA* was also expressed, suggesting a species-specific interaction between AddAB and RecA and also that *H. pylori* AddAB participates in both DNA repair and recombination. These results support a role for both the AddAB nuclease and helicase in DNA repair and promoting infectivity.

Infection of the stomach with *Helicobacter pylori* causes a variety of diseases including gastritis, peptic ulcers, and gastric cancer (1). A central feature of the pathology of these conditions is the establishment of a chronic inflammatory response that acts both on the host and the infecting bacteria (2). Both epithelial (3, 4) and lymphoid (5, 6) cells in the gastric mucosa of infected individuals release DNA-damaging agents that can introduce double-stranded (ds)[2] breaks into the bacterial chromosome (7). The ds breaks must be repaired for the bacteria to survive and establish chronic colonization of the stomach. Homologous recombination is required for the faithful repair of DNA damage and bacterial survival. Alteration of the expression of one of a series of cell surface proteins on *H. pylori* occurs by an apparent gene conversion of *babA*, the frequency of which is reduced in repair-deficient strains (8, 9). This change in the cell surface, which may allow *H. pylori* to evade the host immune response, is a second means by which recombination can promote efficient colonization of the stomach by *H. pylori*.

The initiation or presynaptic steps of recombination at dsDNA breaks in most bacteria involves the coordinated action of nuclease and helicase activities provided by one of two multisubunit enzymes, the AddAB and RecBCD enzymes (10). *Escherichia coli recBCD* null mutants have reduced cell viability, are hypersensitive to DNA-damaging agents, and are homologous recombination-deficient (11–14). Similarly, *H. pylori addA* and *addB* null mutants are hypersensitive to DNA-damaging agents, have reduced frequencies of *babA* gene conversion, and colonize the stomach of mice less efficiently than wild-type strains (8).

The activities of RecBCD enzyme from *E. coli* (15–19) and AddAB from *H. pylori* (8) or *Bacillus subtilis* (20–23) indicate some common general features of the presynaptic steps of DNA repair. In the case of *E. coli*, repair begins when the RecBCD enzyme binds to a dsDNA end and unwinds the DNA using its ATP-dependent helicase activities (17, 24). Single-stranded (ss) DNA produced during unwinding, with or without accompanying nuclease, is coated with RecA protein (16, 25). This recombinogenic substrate engages in strand exchange with a homologous intact duplex to form a joint molecule. Joint molecules are thought to be converted into intact, recombinant DNA either by replication or by cutting and ligation of exchanged strands (26).

Although the AddAB and RecBCD enzymes appear to play similar roles in promoting recombination and DNA repair, they differ in several ways. RecBCD is a heterotrimer, composed of one copy of the RecB, RecC, and RecD gene products (27), whereas AddAB has two subunits, encoded by the *addA* and *addB* genes (21, 28). The enzyme subunit(s) responsible for helicase activity can be inferred from the presence of conserved protein domains or the activity of purified proteins. AddA, RecB, and RecD are superfamily I helicases with six highly conserved helicase motifs, including the conserved Walker A box found in many enzymes that bind ATP (29–32). A Walker A box is defined by the consensus sequence (G/A)*XX*G*X*GKT (*X* is any amino acid (29). RecBCD enzymes in which the con-

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Tables S1–S3.

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[2] The abbreviations used are: ds, double-stranded; ss, single-stranded; HEL, helicase; NUC, nuclease; EOP, efficiency of plating; MIC, minimal inhibitory concentration; CI, competitive index.

served Lys in this motif is changed to Gln have a reduced affinity for ATP binding (33, 34) and altered helicase activity (17, 35–37).

A nuclease domain with the conserved amino acid sequence LDYK is found in RecB, AddA, AddB, and many other nucleases (38). The conserved Asp plays a role in Mg^{2+} binding at the active site; Mg^{2+} is required for nuclease activity (39). The *recB1080* mutation, which changes codon 1080 from the conserved Asp in this motif to Ala, eliminates nuclease activity (39).

We have recently shown that *addA* and *addB* deletion mutants are hypersensitive to DNA-damaging agents and impaired in colonization of the mouse stomach compared with wild-type strains (8). To determine the roles of the individual helicase and nuclease activities of *H. pylori* AddAB in DNA repair and infectivity, we used site-directed mutagenesis to inactivate the conserved nuclease domains of *addA* and *addB* and the conserved ATPase (helicase) domain of AddA. Here, we report that loss of the AddAB helicase is sufficient to impair *H. pylori* DNA repair and infectivity and, when the genes are expressed in *E. coli*, homologous recombination. AddAB retains partial activity in biochemical and genetic assays when either of the two nuclease domains is inactivated but loses all detectable nuclease activity when both domains are inactivated. Remarkably, *H. pylori* AddAB can produce recombinants in *E. coli* only in the presence of *H. pylori* RecA, suggesting a species-specific interaction in which AddAB facilitates the production of ssDNA-coated with RecA protein. Our results show that both the helicase and nuclease activities are required for the biological roles of *H. pylori* AddAB.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, and Growth Conditions—*E. coli* and *H. pylori* strains with their genotypes are listed in Table S1. Plasmids are listed in Table S2. Allele numbers and designations for polypeptides with nuclease (NUC) or helicase (HEL) mutations are indicated as superscripts for clarity. *E. coli* strains were constructed by $CaCl_2$-mediated transformation; fresh transformants were made prior to each experiment. *H. pylori* strains were constructed by natural transformation. *E. coli* and *H. pylori* strains were cultured as described by Amundsen *et al.* (8).

Mutant Construction—Mutations in the nuclease ($addA^{NUC}$ and $addB^{NUC}$) and helicase motifs ($addA^{HEL}$) were introduced into pSA405 (pDuet-1 with *addA* and *addB* (8)) using the QuikChange II XL site-directed mutagenesis kit (Stratagene) according to the manufacturer's instructions. Oligonucleotides (Integrated DNA Technologies) carrying the desired mutations are listed in Table S3. Plasmids with the targeted mutations were identified by sequencing and introduced into *E. coli* strain V3060. Helicase and nuclease motif mutant alleles were introduced into *H. pylori* at the *rdxA* locus by natural transformation (40) of the relevant NSH57-derived deletion strain (8) with derivatives of the pRdxA vector (41) containing the appropriate point mutant gene subcloned from pSA405 derivatives described above. In all cases expression was driven from the endogenous promoter of the *rdxA* locus.

Genetic Assays—Recombination proficiency was measured in crosses as described previously (42) using *E. coli* Hfr donor strain V1306 ($his^+ rpsL^+$; PO44) and F^- recipients (*hisG4 rpsL31*) carrying plasmids expressing *E. coli recBCD*, *H. pylori addAB*, *H. pylori recA*, or combinations of these and lacking *E. coli* chromosomal *recA* or *recBCD* null alleles. Selection was for $His^+ Str^R$.

Cell-free Extract Preparation and Assays—Cell-free extracts were prepared and assayed for helicase and nuclease activities as described (8).

Ciprofloxacin Sensitivity Testing—For *H. pylori*, $\sim 3 \times 10^7$ bacteria were inoculated on horse blood plates. For *E. coli*, $\sim 1 \times 10^7$ bacteria were inoculated on LB agar plates. A single E-test strip (AB Biodisk) was placed in the center of each plate, and plates were incubated at 37 °C for 2–4 days (*H. pylori*) or 1 day (*E. coli*). The minimal inhibitory concentration (MIC) was determined according to the manufacturer's instructions.

Mouse Infections—Female C57BL/6 mice 24–28 days old were obtained from Charles River Laboratories and certified free of endogenous *Helicobacter* infection by the vendor. The mice were housed in sterilized microisolator cages with irradiated PMI 5053 rodent chow and autoclaved corncob bedding, and acidified reverse osmosis-purified water was provided *ad libitum*. All studies were done under the practices and procedures of Animal Biosafety Level 2. The facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International, and all activities were approved by the Fred Hutchinson Cancer Research Institutional Animal Care and Use Committee.

Groups of five mice were infected with *H. pylori* via oral gavage. After 1 week bacteria were recovered, and a competitive index was calculated as described (8).

RESULTS

Construction of addAB Nuclease and Helicase Mutants—To assess the role of the nuclease and helicase activities of AddAB in DNA repair, recombination, and infectivity, we genetically inactivated one or another function. Nuclease and helicase domains were identified by comparing the amino acid sequences of *H. pylori* AddA and AddB with known motifs in *E. coli* RecB and RecD and in *B. subtilis* AddA and AddB (8, 20, 32, 38, 39, 43, 44). To inactivate the putative AddA helicase, we targeted the Walker A box responsible for ATP binding by changing codon 18 from Lys to Gln (K18Q), here designated $AddA^{HEL}$. The nuclease domains of AddA and AddB were identified by the conserved RecB nuclease motif LDYK. To inactivate the nuclease domain, we changed the Asp in this motif to Ala in *addA* (D897A) or in *addB* (D667A), here designated $AddA^{NUC}$ or $AddB^{NUC}$, respectively. Mutations were introduced into the *addAB* genes carried on an *E. coli* expression vector. For analysis in *H. pylori*, mutant alleles were integrated and expressed at the *rdxA* locus, and the endogenous locus was deleted (8, 41).

Both AddA and AddB Have ATP-dependent dsDNA Exonuclease Activity—We assayed nuclease activity, as acid-soluble products of radioactive DNA, in extracts of *E. coli* cells with a chromosomal *recBCD* deletion (Δ*recBCD*) bearing a plasmid encoding the wild-type or mutant *addAB* alleles. Extracts from cells without AddAB (vector control), $AddA^{HEL}B$, and the $AddA^{NUC}B^{NUC}$ extract had very little ATP-dependent nuclease activity, indicating that inactivating both nuclease domains or the single helicase domain eliminates detectable exonuclease activity (Table 1). The $AddA^{HEL}B$ enzyme likely fails to degrade DNA because, as for the RecBCD enzyme, the exonuclease acts almost exclusively during DNA unwinding (45–49). In contrast, inactivating the single nuclease motif in *addA* or in *addB* reduced the nuclease activity to about half that of the wild type (Table 1). This suggests that the individual nuclease domains of AddA and AddB can act independently during DNA unwinding.

We next measured the nuclease activity of mutant and wild-type AddAB enzymes in intact cells. In *E. coli*, the nuclease activity of RecBCD enzyme degrades the DNA of phage T4 lacking the protective gene 2 protein; this restriction greatly reduces the efficiency of plating (EOP) of phage T4 gene *2* mutants (50, 51). In *E. coli* Δ*recBCD* cells, expression of AddAB or $AddA^{NUC}B$ or $AddAB^{NUC}$ reduced the EOP to about 10^{-5} of that with the vector control (Table 1). The EOP was ~1 on cells with $addA^{HEL}$ or $addA^{NUC}B^{NUC}$, indicating that phage plating was not restricted in these cells, consistent with the absence of nuclease activity in assays of the corresponding extracts (Table 1). The strong reduction of EOP on $addA^{NUC}B$ or $addAB^{NUC}$ cells indicates that each of the two nuclease domains has significant nuclease activity, as observed in cell-free extracts (Table 1). Thus, both the intracellular and cell-free extract results suggest that the single nuclease domains can act independently but are dependent on the helicase for detectable nuclease activity.

TABLE 1

Mutations in either the nuclease or helicase domain of AddAB reduce or eliminate exonuclease activity in cells and in cell-free extracts

AddAB plasmid[a]	Codon change	ATP-dependent ds DNA exonuclease activity[b]	Efficiency of plating[c]	
			T4	T4 gene 2 mutant
Vector	NA[d]	17 ± 5	1.0	1.0
AB	None	594 ± 65	0.9	1.8×10^{-5}
$A^{NUC}B$	D897A	210 ± 32	1.1	2.2×10^{-4}
AB^{NUC}	D667A	225 ± 44	0.8	1.1×10^{-5}
$A^{NUC}B^{NUC}$	D897A and D667A	28 ± 6	0.9	0.7
$A^{HEL}B$	K18Q	31 ± 9	1.2	0.8

[a] Plasmids are derivatives of pETDuet-1 encoding the indicated AddAB subunits.

[b] Units/mg extract protein (46). Extracts were prepared from transformants of *E. coli* strain V3060 (Δ*recBCD2731* ⟨kan⟩ DE3) with pETDuet-1 or the indicated derivatives. Cells were harvested 3–5 h after induction with 1 mM isopropyl 1-thio-β-D-galactopyranoside. Data are the mean ± S.E. from at least six independent extracts.

[c] Phage titer on *E. coli* strain V3060 (Δ*recBCD2731* ⟨kan⟩ DE3) with the indicated plasmid divided by the phage titer on strain V3060 with the vector pETDuet-1. Data are the means from two experiments. At least 60 plaques were counted for each determination.

[d] NA, not applicable.

Helicase Activity of addAB Mutants—The AddAB and RecBCD enzymes unwind linear dsDNA in an ATP-dependent reaction. We measured helicase activity under reaction conditions that minimize nuclease activity, with excess ATP relative to Mg^{2+} (45). Cell-free extracts were reacted briefly (2 min) with the ^{32}P end-labeled substrate, and the products were separated by agarose gel electrophoresis and visualized by autoradiography. Extracts containing AddAB, $AddA^{NUC}B$, $AddAB^{NUC}$, or $AddA^{NUC}B^{NUC}$ unwound dsDNA, producing similar amounts of ssDNA products (Fig. 1). As expected, mutation of one or both of the nuclease motifs had no apparent effect on the helicase activity. In contrast, inactivation of the AddA ATP-binding domain ($AddA^{HEL}B$) abolished helicase activity, and no ssDNA was detected even with more than 10 times the amount of extract protein that gave detectable unwinding with the other strains (Fig. 1).

Collectively, the experiments described above demonstrate that site-directed mutations in the nuclease and helicase domains of AddA or AddB altered the expected target activities (Table 1 and Fig. 1). We next determined how loss of these activities affected DNA repair, recombination, and infectivity.

Sensitivity of H. pylori addAB Mutants to DNA-damaging Agents—AddAB plays a central role in DNA repair, and *addAB* null mutants are sensitive to DNA-damaging agents (8, 32). We wished to determine in *H. pylori* whether inactivation of the helicase domain or one or both of the nuclease domains of AddAB increased the sensitivity of cells to the DNA gyrase inhibitor ciprofloxacin. Ciprofloxacin induces ds breaks in the DNA, a target for AddAB-mediated repair (52–54). *H. pylori* strains with chromosomal *addA* or *addB* deletions had a lower MIC of ciprofloxacin than the wild type (Fig. 2).

To compare the phenotype of wild type and nuclease or helicase mutants, *addA* or *addB* alleles (wild type or mutant) were inserted into the chromosomal *rdx* locus, at which inserted genes are often overexpressed compared with the normal chromosomal location (8, 43). *H. pylori s*trains with a mutation in the helicase motif ($addA^{HEL}$) or the nuclease motif of either *addA* or *addB* ($addA^{NUC}$ or $addB^{NUC}$) had a lower MIC of ciprofloxacin than wild-type *addAB* at *rdx* (Fig. 2). These data

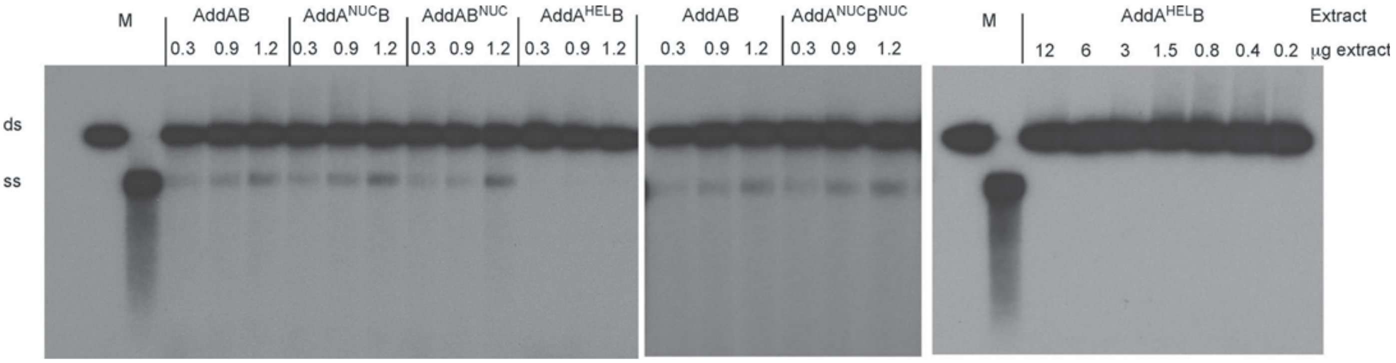

FIGURE 1. **The $AddA^{HEL}$ alteration eliminates DNA unwinding activity, but the $AddA^{NUC}$ and $AddB^{NUC}$ alterations do not.** Extracts were prepared from *E. coli* strain V3060 (Δ*recBCD2731* DE3) carrying derivatives of pSA405 expressing *H. pylori* AddAB proteins as indicated. The indicated amounts of protein were reacted at 37° for 2 min with ^{32}P 5′-end labeled linearized pBR322 in reaction buffer containing 5 mM ATP and 2 mM Mg^{2+}. The products of the reaction were separated by agarose gel electrophoresis and autoradiographed. The positions of the dsDNA substrate (*ds*) and unwound ssDNA (*ss*) are indicated.

suggest that AddAB helicase and nuclease are required for repair of ciprofloxacin-induced damage.

Homologous Recombination in E. coli Requires Both Wild-type H. pylori AddAB and RecA—DNA damage induced by ciprofloxacin is thought to be repaired by homologous recombination between the damaged DNA and an intact duplex. Recombination proficiency can be measured quantitatively in *E. coli* following conjugation with an Hfr strain, which introduces broken DNA into the recipient cells. Because both AddAB and RecBCD have helicase and nuclease activities (Table 1 and Fig. 1), we tested the ability of wild-type and mutant AddAB to promote recombination in *E. coli* recipients lacking RecBCD, an enzyme essential for recombination in *E. coli* Hfr crosses (11, 12, 14).

To measure conjugation, we expressed *H. pylori* AddAB from plasmids in *E. coli hisG rpsL* (His$^-$Str^R) strains with a chromosomal *recBCD* deletion. These strains were mated with an *rpsL*$^+$ Hfr strain that transfers *hisG*$^+$ early, and His$^+$ [Str^R] recombinants were selected (42). The recombinant frequency in the *recB21* null mutant was reduced more than a thousand-fold compared with that in a *recBCD*$^+$ strain (Table 2, compare crosses 1 and 2). Surprisingly, strains expressing wild-type AddAB were as recombination-deficient as *recB21* (Table 2, cross 5). Strains expressing AddA or AddB with altered nuclease or helicase domains were also recombination-deficient (data not shown). This suggests that the nuclease and helicase activities provided by wild-type *H. pylori* AddAB are not able to produce viable recombinants in *E. coli*.

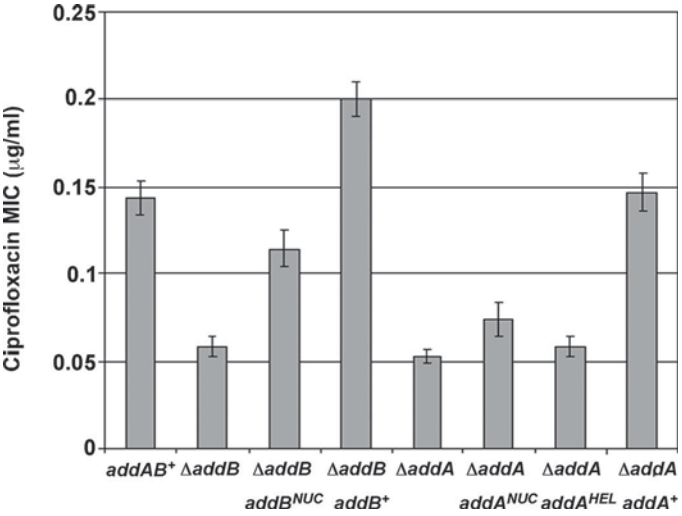

FIGURE 2. **Both AddAB nuclease and helicase activities are required for *H. pylori* wild-type resistance to ciprofloxacin.** The minimal inhibitory concentration of ciprofloxacin for each strain was determined using E-test strips; the mean ± S.E. is indicated ($n = 3–5$). Strains are derivatives of *H. pylori* strain NSH57. The wild-type or mutant NUC or HEL *addAB* allele was integrated at the *rdx* locus, and the corresponding endogenous locus was deleted.

In *E. coli*, RecBCD enzyme appears to interact with RecA protein during the presynaptic stages of homologous recombination, loading RecA onto DNA to produce a recombinogenic ssDNA filament (16); this activity is required to produce recombinants (55). We tested the possibility that AddAB did not support recombination in *E. coli* because it was unable to load *E. coli* RecA protein onto DNA. RecA protein is required for Hfr conjugation (56); the recombinant frequency in *recBCD*$^+$ *E. coli* was reduced more than a thousand-fold in the presence of *recA56*, a *recA* null allele (Table 2, cross 3). When both *H. pylori* AddAB and RecA were expressed in *E. coli*, the recombinant frequency was increased more than 250-fold relative to that in strains with either *H. pylori* AddAB or RecA alone; this increase is statistically significant ($p < 0.0001$) (Table 2, crosses 6, 7, and 9). A similar increase was obtained when *H. pylori recA* was expressed in a *recA56* background with *H. pylori addAB* (Table 2, cross 8). These results indicate that *H. pylori* AddAB and RecA must interact to produce viable recombinants. Such a species-specific interaction could be explained if, like *E. coli* RecBCD, AddAB facilitates the loading of RecA onto ssDNA. The observed specificity was also true for *E. coli* RecBCD: *H. pylori* RecA did not complement a *recA* null mutation (Table 1, cross 6).

Because repair of DNA damage can occur by homologous recombination, we tested whether co-expression of *H.pylori addAB* and *recA* could reduce the sensitivity of an *E. coli recBCD* deletion strain to UV light or to the DNA gyrase inhibitor, ciprofloxacin. Both agents lead to the production of dsDNA breaks. The strains expressing *H. pylori addAB* alone

TABLE 2

H. pylori* AddAB nuclease and helicase activities, as well as *H. pylori* RecA, are required to promote homologous recombination in *E. coli

Cross	*recBCD* or *addAB* allele[a]		*recA* allele[b]		Hfr recombinant frequency[c]
	Chromosome	Plasmid	Chromosome	Plasmid	
					% His$^+$ [Str^R]
1	*recBCD*$^+$		*recA*$^+$		5.6 ± 0.4
2	*recB21*		*recA*$^+$		0.001 ± 0.0008
3	*recBCD*$^+$		*recA56*		0.004, 0.002
4	Δ*recBCD2731*	*recBCD*$^+$	*recA*$^+$		4.3 ± 0.2
5	Δ*recBCD2731*	*addAB*$^+$	*recA*$^+$		0.002 ± 0.0006
6	*recBCD*$^+$		*recA56*	*Hp recA*$^+$	0.002 ± 0.001
7	*recBCD*$^+$	*addAB*$^+$	*recA56*		0.002, 0.001
8	Δ*recBCD2731*	*addAB*$^+$	*recA56*	*Hp recA*$^+$	0.1, 0.1
9	Δ*recBCD2731*	*addAB*$^+$	*recA*$^+$	*Hp recA*$^+$	0.5 ± 0.1
10	Δ*recBCD2731*	*addA*^{NUC}*B*	*recA*$^+$	*Hp recA*$^+$	0.05 ± 0.01
11	Δ*recBCD2731*	*addAB*^{NUC}	*recA*$^+$	*Hp recA*$^+$	0.06 ± 0.01
12	Δ*recBCD2731*	*addA*^{NUC}*B*^{NUC}	*recA*$^+$	*Hp recA*$^+$	0.002 ± 0.003
13	Δ*recBCD2731*	*addA*^{HEL}	*recA*$^+$	*Hp recA*$^+$	0.001 ± 0.0004

[a] Strains are transformants of *E. coli* strain V3060 (Δ*recBCD2731* ⟨kan⟩ DE3) or V3063 (*recA56* DE3) with plasmid pMR3 (*recBCD*$^+$), pSA405 (*addAB*$^+$), or derivatives of pSA405 carrying the indicated *addAB* mutant alleles (see supplemental Table S2).

[b] Strains have the indicated *E. coli* chromosomal *recA* alleles with or without plasmid pSA502 expressing the *H. pylori recA*$^+$ gene.

[c] Data are the mean ± S.E. from 3 to 6 independent matings, except where data from two independent experiments are shown. For cross 9, $p < 0.0001$ compared with cross 5. For crosses 10 and 11, $p < 0.005$ compared with cross 5.

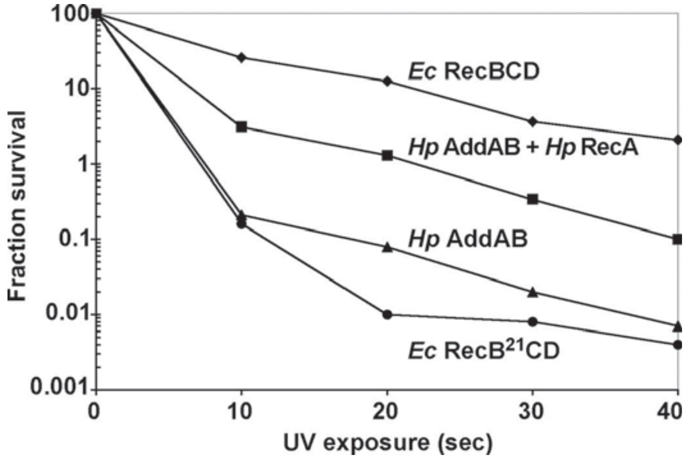

FIGURE 3. ***H. pylori* AddAB and RecA restore partial UV resistance to an *E. coli* Δ*recBCD* strain.** Strains are transformants of *E. coli* strain V3060 with the indicated proteins expressed from plasmids. Cultures were grown to mid-log phase at 37 °C in LB with appropriate antibiotics, harvested by centrifugation, resuspended in 10 mM $MgSO_4$, exposed to UV light, and plated on LB agar plates containing appropriate antibiotics. Survival is the fraction of initial colony-forming units surviving after exposure to the indicated fluence of UV light. The data shown are representative of three independent experiments.

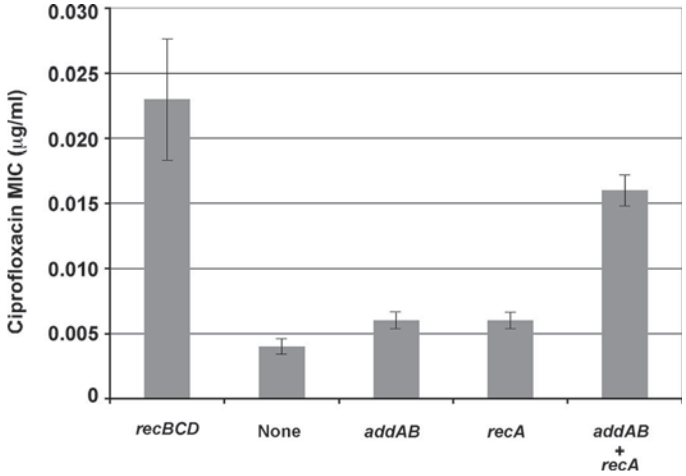

FIGURE 4. ***H. pylori* AddAB and RecA are required to restore partial ciprofloxacin resistance to an *E. coli* Δ*recBCD* strain.** Strains are transformants of *E. coli* strain V3060 with the indicated alleles on plasmids. Cultures were grown to mid-log phase at 37 °C in LB with appropriate antibiotics and spread on LB plates. The minimal inhibitory concentration (mean ± S.E.; $n = 3$) of ciprofloxacin as determined with E-test strips is indicated.

were about as sensitive to UV light as a strain with the *recB* null allele (*recB21*) (Fig. 3). The *E. coli* strain with both *H. pylori* AddAB and *H. pylori* RecA showed greater resistance to UV light, approaching the resistance of the *E. coli* wild type. A similar pattern was observed in the sensitivity to ciprofloxacin (Fig. 4). The MIC for the *E. coli* strain expressing *H. pylori addAB* and *recA* was similar to that for *recBCD*$^+$ *E. coli*. Strains expressing *H. pylori addAB* or *recA* alone were about as sensitive to ciprofloxacin as a *recBCD* null mutant. These data further support a species-specific interaction between AddAB and RecA in DNA repair.

We next tested the role of the AddAB helicase and nuclease activities in promoting Hfr recombination in *E. coli* expressing *H. pylori recA*. The recombinant frequency in strains with a single nuclease domain alteration (AddA^{NUC}B or AddAB^{NUC}) was reduced about 10-fold compared with that with the fully wild-type combination (AddAB with *H. pylori* RecA) but was significantly greater than that in the crosses without *H. pylori* RecA ($p < 0.005$; Table 2, crosses 10 and 11). AddA^{NUC}B^{NUC} and AddA^{HEL}B were as recombination-deficient as a null mutant even in the presence of *H. pylori* RecA (Table 2, crosses 12 and 13). Taken together, these data indicate that both the nuclease and helicase activities of AddAB are required for DNA repair and homologous recombination but that a single nuclease domain in AddAB can provide sufficient activity to produce recombinants, although at a reduced level.

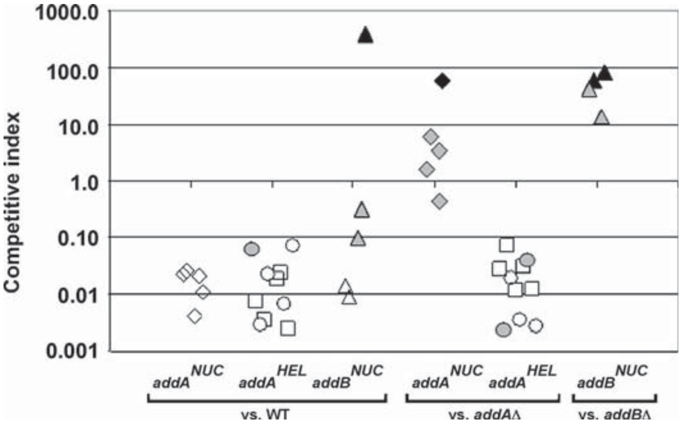

FIGURE 5. **AddAB nuclease and helicase activities are required for wild-type *H. pylori* stomach colonization.** Mice were orally infected with a 1:1 mixture of the indicated *H. pylori* strains. After 1 week the bacteria colonizing the stomach were harvested and the competitive index determined (ratio of nuclease or helicase mutant to deletion or wild type (*WT*) in the output corrected for the input ratio). Each datum is the CI from one mouse. *Open symbols* indicate mice from which only wild-type or deletion mutant bacteria were recovered and represent an upper limit on the competitive index with 95% confidence (Poisson distribution). Similarly, *black symbols* indicate animals in which only the nuclease or helicase mutant was recovered and represent a lower limit on the competitive index with 95% confidence. *Gray symbols* indicate animals where both strains in the inoculum were recovered at 1 week. The results of infections with two different *addA^{HEL}* clones are indicated by *circles* and *squares*. CI < 1 indicates a colonization defect, and CI > 1 indicates out-competition.

H. pylori AddAB Nuclease and Helicase Are Required for Efficient Colonization of the Mouse Stomach—Deletion of *recA*, *addA*, or *addB* reduces the efficiency of *H. pylori* stomach colonization in mice (8), suggesting a role for DNA repair during infection. To further explore the importance of AddAB during infection, we determined the contribution of distinct AddAB biochemical activities to infectivity.

We first tested the ability of *H. pylori* AddAB nuclease or helicase mutants to colonize mice following co-infection with wild-type bacteria. After 1 week, we recovered bacteria from the stomach and calculated a competitive index (CI), the ratio of mutant to wild-type bacteria recovered. A CI of less than 1 for a mutant indicates a colonization defect relative to wild-type. The *addA^{NUC}B* and *addA^{HEL}B* strains had a CI of 0.1 or less in all mice tested, indicating a deficiency comparable with that of *addAB* null mutants (Fig. 5) (8). In fact, no mutant bacteria were recovered from infections with the *addA^{NUC}B* strain; mutant bacteria were recovered in only 1 of 10 mice with the *addA^{HEL}B* mutant. The *addAB^{NUC}* strain demonstrated considerable mouse-to-mouse variation. In four mice the CI was less than 1; two mice yielded no mutant bacteria, but in one mouse the mutant colonized to a higher level than wild-type. These data indicate that in most cases, full AddAB nuclease activity is required to maintain *H. pylori* viability during mouse colonization; the activity provided by a single nuclease domain is not sufficient. The *addA*^{HEL}*B* strain was as deficient as the *addA* null mutant (8) in colonization activity, further demonstrating the role of helicase and nuclease activities in supporting infection (Table 1 and Fig. 1).

We next compared the infectivity of *H. pylori addA*^{NUC}*B*, *addAB*^{NUC} or *addA*^{HEL}*B* to Δ*addA* or Δ*addB*; the deletion strains lack nuclease activity and are deficient in DNA repair and colonization (8). Similarly, extracts of *H. pylori* expressing the AddA or AddB subunit alone lack ATP-dependent dsDNA exonuclease activity, suggesting that both subunits are required for nuclease activity (8). As expected, the partial nuclease activity of *H. pylori addA*^{NUC}*B* or *addAB*^{NUC} allowed more efficient colonization than Δ*addA* or Δ*addB* strains (Fig. 5).

Remarkably, the CI for *addA*^{HEL}*B* relative to Δ*addA* in 10 mice was 0.1 or less, indicating that the helicase mutant was *more* deficient in colonization than a strain that expressed only the AddB subunit (Fig. 5). This suggests that AddA^{HEL}B is stably expressed during infection and that there is an activity remaining in the enzyme that reduces the viability of *H. pylori* during colonization. Under the "Discussion" we have suggested an explanation for this observation. Collectively, these results indicate that the nuclease and helicase activities of AddAB are required to maintain *H. pylori* viability for efficient colonization.

DISCUSSION

Repair of DNA damage is required to maintain viability in all organisms. In pathogenic bacteria such as *H. pylori,* DNA repair is required to reverse host-inflicted damage that occurs as a consequence of the immune response, which releases agents, including reactive oxygen species, that introduce ds breaks into the bacterial chromosome (7). Failure to repair such damage limits bacterial colonization of the host. A central component and initiating agent of the repair pathway of many bacteria is the AddAB or closely related RecBCD enzyme. These enzymes use a complex set of activities to repair DNA damage by homologous recombination. We reported previously that *H. pylori* AddAB promotes DNA repair and is required for efficient colonization of the stomach (8). Here, we used site-directed mutagenesis of functional enzyme motifs to dissect the role of the ATP-dependent ds exonuclease and helicase activities of *H. pylori* AddAB. We found that both activities were required for repair and efficient colonization of the stomach in a mouse model.

Mutations Inactivating the H. pylori AddAB Nucleases and Helicase—The RecB, AddA, and AddB proteins of *E. coli*, *B. subtilis*, and *H. pylori* share the RecB nuclease motif involved in binding Mg^{2+} to the nuclease active site (8, 20, 38, 57, 58). A mutation changing the aspartic acid to alanine in AddA (D897A) and AddB (D667A) together eliminated nuclease activity (AddA^{NUC}B^{NUC}; Table 1). Interestingly, the same codon change in only one or the other subunit reduced, by about half, the specific activity of the corresponding mutant enzymes (Table 1). This indicates that the nuclease domains of AddA and AddB can act independently during DNA unwinding. Yeeles and Dillingham (20) have shown that each subunit of *B. subtilis* AddAB degrades one strand of a DNA duplex. This dual nuclease mechanism is consistent with the data reported here. The observation that an *H. pylori addA* or *addB* deletion strain lacks ATP-dependent nuclease activity (8) indicates, however, that the subunits cannot act alone. We infer that there is a complex interaction of AddAB subunits as there is for the subunits of the *E. coli* RecBCD enzyme (55, 59–65).

The helicase activity of AddAB converts dsDNA to ssDNA in an ATP-dependent reaction. The ssDNA intermediate is thought to invade a homologous duplex following the initiating steps of homologous recombination. Mutations that change a lysine to glutamine in the ATP-binding domain reduce the affinity of ATP binding and alter the activity of other helicases (17, 32–34, 36, 37, 66). Cell-free extracts containing AddA^{HEL}B enzyme failed to unwind DNA, although those containing AddA^{NUC}B, AddAB^{NUC}, or AddA^{NUC}B^{NUC} produced similar amounts of ssDNA as extracts containing AddAB enzyme (Fig. 1). The importance of helicase activity to the overall mechanism of enzyme action is demonstrated by the absence of dsDNA exonuclease activity in AddA^{HEL}B extracts (Table 1). This suggests, as with RecBCD enzyme, that the exonucleolytic degradation occurs during or after DNA unwinding.

Evidence for Species-specific Interactions of RecA and RecBCD or AddAB—Genes encoding a representative of the AddAB and RecBCD classes of enzymes are found in nearly all of the bacterial genomes sequenced (67, 68).[3] This suggests a common need for ATP-dependent exonucleases and helicases in DNA metabolism and raises the possibility that the gene products from different organisms might be interchangeable (10). Clearly, *H. pylori* AddAB has nuclease activity in *E. coli* (Table 1 and Ref. 8), but expression of *addAB* failed to restore recombination proficiency in the same strain (Table 2, cross 5). Partial recombination proficiency in the *recBCD* deletion strain was restored only when *H. pylori* AddAB and RecA were expressed together (Table 2, cross 9). Similarly, recombinants were produced in the *E. coli recA56* null mutant only when *H. pylori* RecA and AddAB were co-expressed (Table 2, cross 8). This indicates a species-specific interaction between AddAB and RecA in two different genetic backgrounds. This result may stem from *H. pylori* AddAB having a third activity demonstrated for RecBCD enzyme: loading RecA protein onto ssDNA as RecBCD unwinds the DNA (16).

The RecA loading activity of RecBCD enzyme plays an essential role in creating the active form of an ssDNA end that undergoes strand exchange with homologous duplex DNA; mutants that fail to load RecA are recombination-deficient (55). Analysis of complexes formed between RecA and the C terminus of RecB protein identified a region that interacts with RecA (69). Similar experiments using RecB and non-cognate DNA strand exchange proteins from *B. subtilis*, *Saccharomyces cerevisiae*, and phage T4 indicate that the efficiency of the interaction compared with that with *E. coli* RecA is reduced (69). The observation that AddAB from *B. subtilis* restores viability and recombination proficiency to an *E. coli recBCD* deletion

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[3] G. Cromie, personal communication.

mutant (70) suggests that *E. coli* RecA interacts to at least a limited degree with *B. subtilis* AddAB. The interaction of *B. subtilis* RecA with *E. coli* RecB outside of cells may parallel the ability to produce recombinants inside cells, although to date there is no reported evidence that *B. subtilis* AddAB actively loads RecA (20).

Additional genetic evidence for a species-specific interaction has been inferred from differences in recombination proficiency observed in *E. coli* when RecBCD and RecA are provided by different species of Enterobacteriaceae (71, 72). Although the relative recombination proficiencies supported by cognate pairs of RecA and RecBCD from *E. coli*, *Serratia marcescens*, and *Proteus mirabilis* in an *E. coli recBCD-recA* deletion mutant varied, the frequency of recombinants was reduced severalfold in the presence of non-cognate pairs (71, 72). Additional work is needed to define the nature of the RecBCD- or AddAB-RecA interaction.

The frequency of recombinants produced by *H.pylori* AddAB and RecA was reduced compared with that of fully wild-type *E. coli* (Table 2; compare cross 8 and 9 to cross 1). This may reflect the level of expression of the *H. pylori* genes in *E. coli*, protein stability, or another factor. One factor that limited RecA protein activity in our experiments may be the reported post-translational modification of *H. pylori* RecA by glycosylation in *H. pylori* but not *E. coli* (73, 74). Expression of *H. pylori* RecA failed to complement the UV sensitivity of an *E. coli recA* mutant (74). Our results (Table 2 and Fig. 3) suggest that expression of *H. pylori addAB* and *recA* together would partially restore the UV resistance of an *E. coli recA* mutant.

Roles of AddAB in H. pylori DNA Damage Repair and Infection—DNA damage from the environment or from cellular activities such as replication or transcription must be repaired for *H. pylori* to infect and successfully colonize the host. The role of AddAB nuclease and helicase activities in DNA repair was tested by determining the sensitivity of *H. pylori* wild-type and mutant strains to the topoisomerase inhibitor ciprofloxacin. DNA lesions resulting from ciprofloxacin treatment include ds breaks, putative entry sites for AddAB. The *addA*^{HEL}*B* strain was as sensitive to ciprofloxacin as *addA* or *addB* deletion strains (Fig. 2). This observation is consistent with the lack of nuclease and helicase activities in the corresponding strains or enzymes (Table 1 and Ref. 8). Inactivation of a single nuclease domain increased sensitivity to ciprofloxacin relative to wild type (Fig. 2), indicating that the helicase and partial nuclease activity remaining in *addA*^{NUC}*B* and *addAB*^{NUC} strains are insufficient to repair DNA damage and maintain cell viability. These data support the importance of AddAB nuclease and helicase activity in maintaining *H. pylori* viability by facilitating DNA repair following host-inflicted DNA damage.

Successful colonization of the mouse stomach by *H. pylori* requires resistance to the host immune response and maintenance of bacterial viability. The importance of AddAB nuclease and helicase activity in this process is indicated by the observation that *addA* and *addB* deletion mutants colonize less efficiently than wild-type (8), *addA*^{NUC}*B*, or *addAB*^{NUC} strains (Fig. 5). The helicase and partial nuclease activities remaining in strains with a single nuclease domain mutation were sufficient to promote more efficient colonization than *addA* or *addB* deletion strains which lack both activities.

Interestingly, *H. pylori addA*^{HEL}*B* demonstrated a greater colonization defect than the *addA* deletion strain (Fig. 5). The mutant AddAB enzymes in both strains lacked detectable nuclease and helicase activity. This suggests that the AddA^{HEL}B mutant enzyme might act upon damaged DNA in such a way that an AddAB-independent repair pathway cannot be used for repair. This pathway would be functional in *addA* deletion strains and contribute to colonization. The *B. subtilis* AddA^{HEL}B mutant enzyme binds tightly to dsDNA ends (20); we infer that the same is true for the *H. pylori* mutant enzyme. AddA^{HEL}B enzyme likely binds to a dsDNA end, blocking accessibility of other repair enzymes. A RecN homolog in *H. pylori* has been described recently (75); its function may be to join dsDNA ends together for repair (76) in an AddAB-independent pathway. Other pathways may also be involved in repair.

It is clear that DNA repair and homologous recombination contribute to the pathogenicity of bacteria in significant ways. DNA damage inflicted by reactive oxygen species generated as part of the immune response or as a consequence of oxidative stress produced by antibiotic treatment (77) must be repaired for bacteria to establish infection. In many organisms, DNA damage induces the SOS response, the expression of a group of genes that promote survival (78). Expression of low fidelity polymerases results in mutagenic DNA repair that can produce mutations that affect virulence or increase resistance to antimicrobial agents (79). SOS induction requires RecBCD in *E. coli* (80, 81), and AddAB may function in the same capacity in other organisms. Establishing the role of the AddAB helicase and nuclease activities in DNA repair, colonization, and the response to a changing host environment will contribute to our understanding of how *H. pylori* efficiently colonizes the stomach and perhaps suggest a target for antimicrobial intervention.

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