Helicobacter pylori AddAB helicase-nuclease and RecA promote recombination-related DNA repair and survival during stomach colonization

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SUMMARY

Helicobacter pylori colonization of the human stomach is characterized by profound disease-causing inflammation. Bacterial proteins that detoxify reactive oxygen species or recognize damaged DNA adducts promote infection, suggesting that *H. pylori* requires DNA damage-repair for successful *in vivo* colonization. The molecular mechanisms of repair remain unknown. We identified homologs of the AddAB class of helicase-nuclease enzymes, related to the *Escherichia coli* RecBCD enzyme, which, with RecA, is required for repair of DNA breaks and homologous recombination. *H. pylori* mutants lacking *addA* or *addB* genes lack detectable ATP-dependent nuclease activity, and the cloned *H. pylori addAB* genes restore both nuclease and helicase activities to an *E. coli recBCD* deletion mutant. *H. pylori addAB* and *recA* mutants have a reduced capacity for stomach colonization. These mutants are sensitive to DNA damaging agents and have reduced frequencies of apparent gene conversion between homologous genes encoding outer membrane proteins. Our results reveal requirements for double-strand break repair and recombination during both acute and chronic phases of *H. pylori* stomach infection.

INTRODUCTION

The Gram(-) bacterium *Helicobacter pylori* chronically infects the stomach of half of the world's human population, causing inflammation in the stomach that can lead to peptic ulcer disease and gastric cancers (Kusters *et al.*, 2006). The host immune system wards off these and other bacteria by exposing them to DNA damaging agents. Bacteria overcome this damage in part by repairing their damaged DNA using homologous recombination. Homologous recombination involves three steps. First, a presynaptic step processes the damaged DNA to produce single-stranded DNA coated with RecA protein. The second, synaptic step involves homology searching and strand exchange promoted

by RecA to produce a joint DNA molecule between the damaged DNA and intact DNA. A third, post-synaptic step results in resolution of the joint molecule or priming of new DNA synthesis.

In *Escherichia coli* the presynaptic step can be catalyzed by two distinct sets of proteins, the heterotrimeric RecBCD complex and the RecFOR proteins, which convert a DNA lesion into a RecA-coated filament (Amundsen and Smith, 2003). It has been proposed that the choice of repair complex depends on whether a double-strand (ds) break (by RecBCD) or a single-strand (ss) break or gap (by RecFOR) must be repaired. Curiously, *H. pylori* and other bacteria in the epsilon branch of Proteobacteria with sequenced genomes were thought to lack many or all components of these complexes due to a failure to identify homologs in their genomes (Rocha *et al.*, 2005). In *H. pylori*, the only convincing, annotated homologs of presynaptic proteins are RecJ and RecR. This is in spite of the facts that *H. pylori* contains RecA (Schmitt *et al.*, 1995, Thompson and Blaser, 1995) and post-synaptic proteins (Tomb *et al.*, 1997, Loughlin *et al.*, 2003), has a population genetic structure indicative of a high amount of recombination between strains (Suerbaum *et al.*, 1998, Falush *et al.*, 2003), and uses gene conversion to vary expression of surface proteins during the course of infection (Solnick *et al.*, 2004).

Recombination and DNA repair initiated at DNA ds breaks in *E. coli* requires the RecBCD enzyme, a heterotrimer composed of one copy of the products of the *recB*, *recC,* and *recD* genes (Taylor and Smith, 1995). The enzyme is an ATP-dependent ds and ss exonuclease, a ss endonuclease, an ATPase, and a highly processive helicase. RecBCD binds tightly to ds DNA ends and initiates unwinding using the fast RecD helicase and the slower RecB helicase (Taylor and Smith, 2003). Although the single nuclease domain resides in RecB (Yu *et al.*, 1998), all three subunits and ATP are required for substantial nuclease activity, because DNA hydrolysis occurs during ATP-dependent DNA unwinding (Amundsen *et al.*, 1986). When the enzyme interacts with a Chi site (5’ GCTGGTGG 3’) during unwinding, a 3’-terminated ss DNA end is produced, onto which RecBCD loads RecA protein (Smith *et al.*, 1981, Ponticelli *et al.*, 1985, Anderson and Kowalczykowski, 1997). This

73 presynaptic filament is the substrate for subsequent steps in recombination or repair with an intact
74 duplex.

75 Activities similar to those of RecBCD enzyme are also found in the two subunit AddAB
76 bacterial enzyme, most extensively studied from *Bacillus subtilis*. Like RecBCD, AddAB has nuclease
77 and helicase activities, both of which are ATP-dependent (Kooistra *et al.*, 1988) and can produce the
78 3'-terminated ss DNA end required for presynaptic filament formation and recombination (Chedin and
79 Kowalczykowski, 2002). Both the AddA and AddB proteins have nuclease domains, while apparently
80 active helicase motifs are found only in AddA (Kooistra *et al.*, 1997, Yeeles and Dillingham, 2007).
81 Thus, although the structure of AddAB differs from that of RecBCD, both enzymes contain ATP-
82 dependent nuclease and helicase activities.

83 Here we demonstrate that *H. pylori* and, by homology, other episilon Proteobacteria do have
84 ATP-dependent nuclease and helicase activities, which, as in most Gram(+) bacteria and some Gram(-)
85 bacteria (Rocha et al., 2005), are encoded by *addA* and *addB* genes. We show that AddAB and RecA
86 are required for efficient colonization of the stomach of mice, RecA more than AddAB. We also
87 demonstrate that AddAB and RecA promote a gene conversion-like event that modulates surface
88 expression of a bacterial adhesin. These results suggest that recombination plays multiple roles during
89 infection: recombination-related repair of DNA damage encountered during infection as well as
90 remodeling of the bacterial surface that may allow evasion of adaptive immune responses or altered
91 bacterial tropism.

92

93 ## RESULTS

94

95 ### Identification of *H. pylori addAB* Homologs

No genes in either of two sequenced *H. pylori* genomes available on the NCBI website (http://www.ncbi.nlm.nih.gov/) were annotated as either *recB* or *addA*. Two BLAST searches against *H. pylori* using the RecB sequence of *Escherichia coli* K12 or and the AddA sequence of *Bacillus subtilis subtilis* identified a group of three proteins with significant E values (from 8×10^{-7} to 4×10^{-16}) in *H. pylori* strains J99 and 26695. Both AddA and RecB consist of a highly conserved helicase domain and a highly conserved nuclease domain. The helicase domain is also found in a group of related helicases including Rep, UvrD, and PcrA. In neither *H. pylori* strain J99 nor strain 26695 did the highest scoring alignments to RecB or AddA include their nuclease domains, suggesting that these *H. pylori* proteins might not be homologs of AddA or RecB but instead might be related helicases.

To identify more likely *H. pylori* AddA or RecB proteins, the group of three high-scoring helicases from each strain was searched for conserved domains against the NCBI Conserved Domain Database (CDD). For both of strains J99 and 26695 only one protein showed a significant alignment to the RecB profile (COG1074); these alignments included both the helicase and nuclease domains of RecB. These two proteins (HP1553 from strain 26695 and jhp1446 from strain J99) were not those with the maximum scoring BLAST results but were 93% identical to each other. The J99 protein was previously annotated as PcrA. Each of the two protein sequences bears a RecB domain corresponding to essentially the full length of the protein sequence. To classify these proteins as AddA or RecB they were BLASTed against the TIGR profile database (http://tigrblast.tigr.org/web-hmm/), which identified them as AddA, rather than RecB, sequences.

No significant hits were obtained in a BLAST search using the *Bacillus subtilis* AddB sequence against *Helicobacter* species. This is likely because AddB proteins consist of a large, poorly-conserved RecC-like "inactivated helicase" domain and a short, well-conserved, RecB-like nuclease domain. Therefore, the highly conserved AddB nuclease motif "GRIDRID" was used to identify the *H. pylori* AddB homologs. One perfect match to this sequence was identified in both strains 26695 and J99 (proteins HP1089 and jhp0336, respectively). These proteins are 94% identical and include an

121 "inactivated superfamily I helicase" domain. Both were described as hypothetical proteins, without an
122 assigned function. Alignment of these proteins to *B. subtilis* AddB showed conservation of the
123 nuclease domain (Figure 1). Interestingly, the HP0275 protein from strain 26695, which was annotated
124 as *addB*, lacks the inactivated helicase domain annotation and does not show significant alignment to
125 the conserved nuclease sequence.

126 Using the criterion of reciprocal best hit by BLAST with the *H. pylori* 26695 AddAB
127 sequences, we identified highly related proteins in all of the sequenced epsilon Proteobacteria,
128 suggesting that these bacteria all contain AddAB. As shown in Figure 1, the nuclease domains of the
129 epsilon Proteobacteria homologs of both AddA and AddB are highly conserved. Unlike *B. subtilis*
130 AddB (Kooistra and Venema, 1991), *H. pylori* AddB does not contain a detectable Walker A box,
131 which is often involved in ATP hydrolysis. This motif is found in AddB of some, but not all, firmicute
132 species. Many Gram (+) and essentially all Gram (-) AddB sequences lack this motif. While *addA* and
133 *addB* are adjacent in the chromosome in most bacteria, including other epsilon Proteobacteria, this is
134 not the case in *H. pylori*. Both genes that we identified as *addA* and *addB* are considered core genes
135 that are not strain variable, since they were observed in 56 *H. pylori* clinical isolates from around the
136 world (Gressmann *et al.*, 2005).
137

138 ## *H. pylori* AddAB Has ATP-dependent Nuclease and Helicase Activity

139 The defining characteristic of AddAB and RecBCD enzymes is ATP-dependent DNA exonuclease
140 activity; this nuclease is apparently active only during DNA unwinding, which requires ATP-
141 hydrolysis. Thus, we measured this activity in wild-type, mutant, and complemented *H. pylori* NSH57
142 strains; the latter have the $addA^+$ or $addB^+$ gene inserted into the chromosomal *rdxA* locus, often used
143 for this purpose (Smeets *et al.*, 2000). As shown in Table 1, cytosolic extracts from wild-type bacteria
144 showed detectable ATP-dependent nuclease activity with ds DNA substrate under conditions

optimized for the *H. pylori* enzyme (supplemental Figure S1). Replacement of either the *addA* or *addB* gene with an antibiotic resistance cassette to create deletion (null) alleles abolished activity. ATP-dependent nuclease activity could be restored in these strains by complementation with $addA^+$ or $addB^+$. As a further control, we showed that disruption of two other recombination genes, *recA* and *ruvC,* had no effect on ATP-dependent nuclease activity.

To determine if *addA* and *addB* are the structural genes sufficient to confer ATP-dependent nuclease activity, we expressed these proteins in an *E. coli* strain deleted for *recBCD.* Cytosolic extracts of this strain without the *addA* and *addB* genes showed a very low level of ATP-dependent nuclease activity that was not enhanced by introduction of the vector control (Table 2). Introduction of an *addAB* co-expression construct (pETDuet-1 *addA addB*) resulted in a >40-fold increase in ATP-dependent nuclease activity. The non-inducible $recBCD^+$ control plasmid (pMR3) resulted in a 10-fold increase in activity.

For an intracellular measure of *H. pylori* AddAB nuclease activity in these *E. coli* cells, we examined the ability of the *addAB* genes to restrict T4 phage infection. The T4 gene *2* protein blocks RecBCD-dependent degradation of the phage DNA upon infection, perhaps by binding to the DNA ends in the virion (Oliver and Goldberg, 1977). A T4 gene *2* mutant can productively infect an *E. coli* strain lacking RecBCD nuclease activity (Amundsen *et al.*, 1990) but shows a six-log reduction in plating efficiency in $recBCD^+$ *E. coli* (Table 2). Similarly, expression of *addAB* in the *recBCD* deletion strain efficiently restricted T4 2^- infection. Thus, the *H. pyori addAB* genes confer nuclease activity both in cell-free extracts and in intact cells.

In addition to being an ATP-dependent ds DNA exonuclease, RecBCD enzyme is a highly processive DNA helicase (Taylor and Smith, 1980). We tested extracts of *E. coli recBCD* mutant cells expressing *H. pylori addAB* or *E. coli recBCD* for unwinding activity using linearized, 5'end-labeled pBR322 DNA as substrate. These extracts unwound linear DNA, but extracts with the vector (pETDuet-1) lacking the *addA* and *addB* genes did not (Figure 2). Unwinding activity by AddAB was

ATP-dependent but slightly weaker than that by RecBCD. These results confirm that the *H. pylori* *addA* and *addB* genes encode an ATP-dependent helicase-nuclease similar to RecBCD enzyme of *E. coli.* In the absence of ATP the effective Mg^{2+} concentration is elevated and ATP-independent nucleases present in the extract degraded some of the substrate to oligonucleotides (Figure 2).

addAB and *recA* Mutants Are Hypersensitive to DNA Damaging Agents

Mutants lacking *E. coli* RecBCD or *B. subtilis* AddAB show increased sensitivity to several antibiotics that damage DNA (Alonso *et al.*, 1993), as do *recA* mutants of several species including *H. pylori* (Schmitt et al., 1995, Thompson and Blaser, 1995). As expected, *H. pylori addA* and *addB* mutant strains also showed heightened sensitivity to the alkylating agent mitomycin C and the DNA gyrase inhibitor ciprofloxacin (Table 3), both of which lead to DNA ds breaks (Iyer and Szybalski, 1963, Wolfson and Hooper, 1985, Sioud and Forterre, 1989). The sensitivity observed for the *addA* and *addB* mutants was similar to that seen for a *recA* mutant and could be complemented by expression of the corresponding gene. We conclude that *H. pylori* AddAB is required for repair of intracellular ds breaks.

In contrast, when we examined UV sensitivity, *addA* and *addB* mutants were markedly less sensitive than a *recA* mutant (Figure 3), suggesting that ds break repair does not play a major role in repair of this damage in *H. pylori*. The modest UV sensitivity of the *addA* and *addB* mutants could be complemented by the corresponding genes. This complementation was particularly evident at the 4 kJ/m^2 exposure, where the complemented strains were slightly more resistant than wild type. This enhanced resistance may result from a higher-than-wild-type level of expression when the genes are at the *rdxA* locus, which shows constitutive high level expression of several proteins (D. M. Pinto-Santini, L. K. Sycuro, N. R. Salama, unpublished observations).

We also queried the role that *H. pylori* AddAB might play in homologous recombination during natural transformation with a chromosomal marker. While the *recA* mutant completely lost the ability to undergo natural transformation, as reported previously (Schmitt et al., 1995, Thompson and Blaser, 1995), there was no measurable difference in transformation efficiency for either the *addA* or *addB* mutant (unpublished data). Thus, while *H. pylori* AddAB does appear to play an important role in repair of certain types of DNA damage, it does not appear to participate in homologous recombination during natural transformation.

AddAB Enzyme and RecA Protein Are Required for Optimal Stomach Colonization

H. pylori proteins that neutralize reactive oxygen species, such as superoxide dismutase (Seyler *et al.*, 2001) and catalase (Harris *et al.*, 2003), promote stomach colonization. Similarly endonuclease III, a protein involved in recognition and processing of oxidized DNA, promotes stomach colonization (O'Rourke *et al.*, 2003), suggesting that *H. pylori* DNA experiences oxidative damage during infection. A possible role for recombination-based repair during infection was suggested by the observation that a mutant lacking a Holliday junction resolvase homolog RuvC had persistence defects during stomach colonization (Loughlin et al., 2003). Therefore, we investigated the role that AddAB and RecA, proteins whose homologs promote the early steps of recombination-based repair, might play in stomach colonization.

We first performed competition experiments by oral infection with 1:1 mixtures of mutant and either wild type or complemented mutant in the NSH57 strain background. We allowed the infection to continue for one week and then harvested the bacteria from the stomachs. Plating on selective and non-selective media allowed enumeration of mutant and total bacteria. A competitive index was computed for each animal as the ratio of mutant to wild-type (or complemented mutant) bacteria recovered after one week, adjusted for the ratio of strains in the inocula. While there is considerable mouse-to-mouse

variation in the assay, the average competitive index of either *addA* or *addB* mutants in competition with either wild type or the complemented mutant was below 1, indicating a colonization defect for the mutants (Figure 4). Curiously, when the *addA* complemented strain was competed with wild type, the competitive index was above 1. As described above, expression of *addA* from the *rdxA* locus may result in a higher expression level that is protective under some circumstances. We were unable to complement the *recA* mutant clone because of the requirement of RecA for natural transformation. However, two independently generated *recA* mutant clones both yielded even lower competitive indices than *addA* or *addB* mutants (Figure 4). While we recovered at least some mutant bacteria in 5 of 8 *addA* mutant and 5 of 9 *addB* mutant competitions with wild type or complemented strains, we never recovered *recA* mutant bacteria in competition experiments. All three mutants (*recA, addA,* and *addB*) showed comparable growth to wild type during *in vitro* culture (supplemental Figure S2).

In order to gain further insight into the infection potential of our *H. pylori* strains we infected groups of five animals with decreasing titers of individual strains to determine the dose required for detectable infection of 50% of the animals (ID_{50}) (supplemental table S4). In this experiment the ID_{50} was 2.3×10^4 bacteria for the wild-type strain, 2.0×10^7 for the *addA* mutant strain, and greater than 2.4×10^{10} for the *recA* mutant strain. We recovered bacteria from only a single animal at a high infecting dose of *recA* mutant bacteria. These results mirror the data from the competition experiments showing significantly attenuated colonization by strains lacking AddAB activity and essentially no colonization by strains lacking RecA.

Recombination Proteins Promote Apparent Gene Conversion at the *babA* Locus

As mentioned above, previous work demonstrated that mutants lacking RuvC, a protein required for resolving recombination intermediates, also have partially attenuated stomach persistence (Loughlin et al., 2003). Interestingly, further studies suggested that RuvC function and, by inference, recombination

facilitate bacterial immune evasion by altering the adaptive immune response (Robinson *et al.*, 2005). The mechanisms by which the immune system becomes redirected remain obscure. We previously showed that *H. pylori* can abolish *babA*-dependent adhesion by a gene conversion-like event between *babA* and a related locus, *babB,* and that this event is selected during infection of some hosts (Solnick et al., 2004). We investigated whether this gene conversion event requires recombination protein activity. To do this, we created null alleles of *recA*, *addA,* and *ruvC* in the J166 strain background where we had previously measured spontaneous *babA* to *babB* gene conversion during *in vitro* culture (Solnick et al., 2004). Real-time PCR was used to quantify the frequency of *babB* at the *babA* locus in bacterial populations expanded from single colonies *in vitro*. The wild-type J166 strains showed a frequency of 3×10^{-5}, corresponding to an approximate rate of gene conversion of 3×10^{-6} per cell division (Lea and Coulson, 1949). As shown in Figure 5, loss of AddA or RecA significantly reduced the frequency of the *babA* to *babB* gene conversion. In contrast loss of RuvC only slightly reduced the frequency of gene conversion, and this difference was not statistically significant. The limit of detection of our assay for the frequency of convertants is approximately 4×10^{-7}. These results suggest that this gene conversion event frequently occurs by a RecA-dependent mechanism that may involve a ds break, since it is AddAB-dependent.

DISCUSSION

We report here that the DNA double-strand (ds) break repair enzyme AddAB and the homologous recombination-promoting protein RecA are required for high-level infection by *H. pylori*. AddAB is a functional homolog of the RecBCD enzyme of *E. coli, Salmonella enterica* serovar Typhimurium, and *Neisseria gonorrhoea*. The *E. coli* enzyme is crucial for repair of DNA breaks and genetic recombination involving linear DNA (Smith, 2001), and the *recC* gene appears to be under positive

selection in uropathogenic strains of *E. coli* (Chen *et al.*, 2006). *N. gonorrhoeae recB*, *recC,* and *recD* mutants are more sensitive than wild-type strains to hydrogen peroxide (Stohl and Seifert, 2006). We discuss below the properties of *H. pylori* AddAB enzyme and its role in colonization of the stomach of mice and compare its role with that of RecA protein, which is also required for DNA repair as well as homologous recombination.

We identified the *addA* and *addB* genes using BLAST searches that started with a consensus sequence for RecB. This polypeptide contains both the canonical seven helicase motifs (Bork and Koonin, 1993) and a nuclease domain (Yu et al., 1998). Although the helicase motifs are highly conserved among a large group of helicases with highly divergent cellular functions, the nuclease domain appears to be unique to RecB-related polypeptides. This dual criterion in our searches may account for our finding the RecB-related polypeptide AddA of *H. pylori*, whereas previous searches were not successful. The AddB polypeptide also contains a closely related nuclease domain and a large region with only scant similarity to helicases. These properties allowed us to identify *H. pylori* AddB. Identification of the *H. pylori addA* and *addB* genes allowed us to discover further that AddAB proteins are in fact well conserved among all the sequenced epsilon Proteobacteria in spite of the fact they have not been annotated in most sequencing projects (Rocha et al., 2005)

Although the *addA* and *addB* genes of many groups of bacteria are adjacent and appear to form an operon, the *H. pylori addA* and *addB* genes are not; they are separated by approximately 500 kb. We suppose that the AddA and AddB polypeptides act together in a complex, as do the RecBCD polypeptides and AddAB polypeptides of other bacteria investigated (Kooistra and Venema, 1991, Taylor and Smith, 1995). As noted below, the phenotypes of *H. pylori addA* and *addB* mutants are indistinguishable, as expected if the polypeptides act in a complex. If so, the control of the unlinked *addA* and *addB* genes to maintain the proper stoichiometry of the two polypeptides remains an interesting question.

By assaying extracts of *H. pylori*, we detected an ATP-dependent nuclease (Table 1 and Figure S1), the defining characteristic of the RecBCD class of enzymes, also called exonuclease V. The ATP-dependence of the nuclease activity is a consequence of DNA degradation occurring only during unwinding, which requires the energy of ATP hydrolysis. ATP-dependent nuclease activity was undetectable in *addA* and *addB* mutants; as expected, this loss was complemented by insertions of the corresponding genes at a distant locus (Table 1). These genes also conferred ATP-dependent nuclease and ATP-dependent DNA unwinding activity to an *E. coli recBCD* deletion mutant (Table 2 and Figure 2), indicating that they are the structural genes for this enzyme. Activity was detected both in extracts and in intact cells, which blocked the growth of a phage T4 mutant lacking a protein that protects linear DNA from nuclease digestion. Expression of these genes in *E. coli* provides a way to make large amounts of *H. pylori* AddAB enzyme for further analysis.

Our phenotypic analyses suggest that, like the *E. coli* RecBCD enzyme, *H. pylori* AddAB functions to repair DNA damage that results in ds breaks; both *addA* and *addB* mutants were highly sensitive *in vitro* to the alkalating agent mitomycin C and the topoisomerase inhibitor ciprofloxacin but only slightly sensitive to UV-induced damage (Table 3). While *addA* and *addB* mutants grow well *in vitro* in the absence of overt DNA damage (Figure S2), these strains have a lower colonization potential in both competition experiments and during single-strain infections (Figure 4, supplementary table S4 and data in Results). This may result from DNA damage specifically encountered in the host environment.

Previous studies of enteric pathogens revealed a role for recombinational repair proteins but apparently not recombination *per se* during infection. *Salmonella enterica* serovar Typhimurium *recBC* mutants are severely attenuated for infection and killing, but suppressors that restore recombinational repair by activating the RecFOR pathway of homologous recombination do not suppress the *in vivo* defects (Buchmeier *et al.*, 1993, Cano *et al.*, 2002). Moreover, *recA* mutants, which essentially lack homologous recombination, show a milder phenotype than *recBC* Salmonella

mutants, and loss of RecA does not impact colonization by the *E. coli* extracellular pathogens EHEC or UPEC (Fuchs *et al.*, 1999). Based on double mutant analyses, the essential *in vivo* function of RecBCD enzyme during Salmonella infection appears to be restoration of stalled replication forks (Schapiro *et al.*, 2003), not recombination. In contrast, our results with *H. pylori*, reported here, indicate a direct role for homologous recombination in stomach infection.

Our results show that two enzymes, AddAB and RecA, which process damaged DNA, enhance the ability of *H. pylori* strains to colonize the stomach (Figure 4 and lower ID_{50}). In contrast to the *Salmonella* results, loss of *H. pylori* RecA causes even more severe attenuation of stomach colonization than loss of AddAB. This result suggests that *H. pylori* experiences a different spectrum of DNA damage during infection than that encountered by Salmonella, and that this spectrum includes ds breaks. Furthermore, recombination functions are essential either to repair DNA damage or for other recombination protein-mediated processes early in *H. pylori* infection.

Unlike Salmonella, *H. pylori* primarily remains extracellular where it is not exposed to phagosome-specific host defenses, although recent studies suggest that a small sub-population of *H. pylori* do reside in an intracellular niche primarily in epithelial cells (Amieva *et al.*, 2002, Aspholm *et al.*, 2006, Necchi *et al.*, 2007). The different cellular and tissue tropisms of these bacteria may account for the different recombination-protein requirements for successful infection of Salmonella and *H. pylori.* Several lines of evidence suggest that even in its extracellular niche, *H. pylori* is exposed to oxidative damage soon after infection. Recent work using the mouse model showed *H. pylori*-dependent infiltration of neutrophils and macrophages one and two days post-infection which then decreased to low levels at three and ten days post-infection, suggesting a rapid innate immune response to infection that is then down-regulated (Algood *et al.*, 2007). Because neutrophil infiltration is a hallmark of human *H. pylori* infection, the interaction of *H. pylori* and cultured neutrophils has been studied in some detail. Interestingly, while *H. pylori* is readily taken up by neutrophils and is a potent activator of the phagosome NADPH oxidase, active flavocytochrome b_{558} complex does not assemble

339 on the *H. pylori*-containing phagosome and instead is redirected to the plasma membrane, leading to
340 extracellular superoxide accumulation (Allen *et al.*, 2005). *H. pylori* also induce both macrophages
341 (Chaturvedi *et al.*, 2004) and epithelial cells (Xu *et al.*, 2004) to produce extracellular hydrogen
342 peroxide by stimulating polyamine oxidase. Finally, isolated gastric pit cells express the phagosome
343 NADPH oxidase components at the plasma membrane and show measurable constitutive extracellular
344 superoxide production that is further induced after exposure to *H. pylori*-derived LPS (Teshima *et al.*,
345 1999). These innate immune responses likely contribute to host cellular damage that may benefit *H.*
346 *pylori* but also necessitates bacterial mechanisms to combat oxidative damage to its DNA, proteins and
347 lipids. AddAB appears to play such a role for *H. pylori.*

348 Loss of several *H. pylori* proteins shown or annotated to recognize DNA damage cause lower
349 colonization loads or decreased persistence of *H. pylori* strains in the mouse model, suggesting that the
350 bacteria experience DNA damage stresses during infection. These proteins include HP0585, a homolog
351 of *E. coli* endonuclease III, which repairs oxidized pyrimidine residues (O'Rourke et al., 2003), MutS2,
352 which in *H. pylori* recognizes and binds 8-oxoguanine (Wang *et al.*, 2005), two DNA glycosylases
353 (Baldwin *et al.*, 2007), and a putative RecN homolog (Wang and Maier, 2008). In *E. coli* and *B.*
354 *subtillis,* RecN promotes RecBCD (AddAB)-dependent ds break repair under some stress conditions
355 and is recruited to large damage foci in the absence of ds break repair (Meddows *et al.*, 2005, Sanchez
356 *et al.*, 2006) Thus, *H. pylori* RecN may interact with AddAB to promote ds break repair during
357 stomach colonization.

358 Our *in vitro* data suggest that *H. pylori* AddAB, like *E. coli* RecBCD, functions specifically at
359 ds breaks. Mutants with loss of AddAB or RecA show equivalent sensitivity to chemicals leading to ds
360 breaks, but the *recA* mutant is much more sensitive to UV exposure than *addAB* mutants (Table 3,
361 Figure 3). These results suggest that an additional RecA-dependent pathway operates in *H. pylori* to
362 repair damage induced by UV. A likely candidate is an analog of the *E. coli* RecFOR pathway. A *recR*
363 homolog has been annotated in the *H. pylori* genome (Tomb et al., 1997). The more severe stomach

364 colonization phenotype of *recA* mutants than of *addAB* mutants (supplemental table S4) may result
365 from an additional requirement for the RecFOR DNA repair pathway during infection. The role of
366 RecA in competence for natural transformation may also contribute to the more severe attenuation of
367 this mutant. Unlike other bacteria, such as *Rhizobium*, *Bacillus*, and *Neisseria* (Haijema *et al.*, 1996,
368 Mehr and Seifert, 1998, Zuniga-Castillo *et al.*, 2004), *H. pylori* AddAB does not appear to contribute
369 to competence, while RecA is absolutely required (Schmitt et al., 1995). Two studies have suggested
370 that competence contributes to stomach colonization, even at early time points (Kavermann *et al.*,
371 2003, Baldwin et al., 2007). Natural transformation is thought to contribute to genetic diversification of
372 the *H. pylori* population later in infection by allowing new alleles to spread through the population via
373 recombination (Suerbaum and Josenhans, 2007). Multiple mutant analyses involving *addAB, recR,* and
374 *com* (DNA transformation competence) genes should begin to address the relative importance of these
375 pathways during infection.

376 RecA and AddAB may also contribute to long-term adaptation to the host environment. We
377 discovered a role for RecA and AddAB in promoting gene conversion between two outer membrane
378 protein (OMP) genes, *babA* and *babB* (Figure 5). *H. pylori* genomes encode a large number of OMPs
379 (60), some of which have been annotated as porins, adhesins or outer membrane transporters. Subsets
380 of OMPs have been grouped into paralogous families suggested to be at least partially redundant (Alm
381 *et al.*, 2000). The largest family of OMPs is the Hops which include the Lewis B blood group antigen-
382 binding adhesin BabA and two highly related Hops BabB and BabC (Alm et al., 2000, Hennig *et al.*,
383 2006). Gene conversion via conserved 5'- and 3'-terminal sequences in *babB* or *babC* can eliminate
384 *babA* adhesin-gene expression. Loss of *babA* expression has been observed in a majority of clones
385 from the infecting bacterial population after the initial colonization event (between four and eight
386 weeks post infection) during experimental monkey infection (Solnick et al., 2004), and BabA can be
387 encoded by one or more of the three *bab* loci (Solnick et al., 2004, Colbeck *et al.*, 2006, Hennig et al.,
388 2006). Loss of BabA protein from the cell surface may result in an altered immune response or,

alternatively, modify bacterial tropism by changing host receptor-binding interactions. In *Neisseria*, pilin antigenic variation is mediated by a RecA-dependent gene conversion event that also requires the RecFOR complex (Mehr and Seifert, 1998) or the RecBCD complex (Hill *et al.*, 2007), depending on the strain background. While an engineered gene conversion event in *H. pylori* is RecA-dependent (Pride and Blaser, 2002), the requirement for RecA and other recombination proteins for *bab* locus conversion had not been examined. We find that the *babA* to *babB* gene conversion event significantly depends on RecA and AddAB (Figure 5). If AddAB, like RecBCD, depends on a DNA end for activity, this result suggests that the mechanism of this conversion involves a ds break.

Colonization by *H. pylori* of its host for decades is required for development of disease. The data presented here suggest that recombinational repair proteins, including AddAB and RecA, play multiple roles during infection. The *in vivo* phenotypes we report here after one week of infection likely result from the requirement for these proteins to combat DNA damage stress induced soon after infection. Interestingly, while *recA* mutants have a very severe phenotype, *addAB* mutants can still colonize. This outcome will allow study of genetic diversification during long-term colonization to further dissect additional roles of recombinational repair proteins during infection. Recombinational repair has been suggested as a target that could enhance the efficacy of other antibiotics that lead to intracellular oxidative stress (Kohanski *et al.*, 2007). Our work suggests that for *H. pylori* and perhaps other bacteria AddAB could be a promising direct target for a novel antimicrobial drug, since this class of enzymes is widely distributed in prokaryotes but not in eukaryotes.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Growth

E. coli strains (Table S1) were grown in media containing Difco tryptone and yeast extract (LB), Terrific Broth (Fisher), or Difco tryptone (TB). These media, phage suspension medium (SM), and top agar have been described (Cheng and Smith, 1989). *H. pylori* strains (Table S1) were grown on solid horse blood agar (HB) plates containing 4% Columbia agar base (Oxoid), 5% defibrinated horse blood (HemoStat Labs), 0.2% ß-cyclodextrin (Sigma), vancomycin (Sigma; 10 μg/ml), cefsulodin (Sigma; 5 μg/ml), polymyxin B (Sigma; 2.5 U/ml), trimethoprim (Sigma; 5 μg/ml), and amphotericin B (Sigma; 8 μg/ml) at 37°C either under a microaerobic atmosphere generated using a CampyGen sachet (Oxoid) in a gas pack jar or in an incubator equilibrated with 14% CO_2 and 86% air. For liquid culture, *H. pylori* was grown in Brucella broth (Difco) containing 10% fetal bovine serum (BB10; Invitrogen) with shaking in a gas pack jar with a CampyGen sachet. For antibiotic resistance marker selection, bacterial media were additionally supplemented with ampicillin (100 μg/ml), kanamycin (50 μg/ml), chloramphenicol (Cm; 15 μg/ml) or metronidazole (Mtz; 36 μg/ml). When culturing bacteria from mouse stomachs, Bacitracin (Bac; 200 μg/ml) was added to eliminate normal mouse microbiota contamination.

DNA Manipulations

DNA manipulations, such as restriction digestion, PCR, and agarose gel electrophoresis, were performed according to standard procedures (Ausubel *et al.*, 1997). Genomic DNA was prepared from *H. pylori* by Wizard genomic DNA preparation kits (Promega). Primers used for PCR and sequencing are in Table S2, and plasmids used in this study are in Table S3.

Generation of *H. pylori* Isogenic Knockout Mutants

In the NSH57 strain background, null alleles of *addA, addB, recA,* and *ruvC* were constructed using a vector-free allelic replacement strategy to generate alleles in which a chloramphenicol acetyl

transferase resistance cassette replaced 80-90% of the coding sequence of the gene while preserving the start and stop codons (Chalker *et al.*, 2001, Salama *et al.*, 2004). The primers used for this procedure are in Table S2. The resistance cassette contains its own promoter but lacks a transcriptional terminator and in all cases was inserted in the same direction of transcription as that of the native gene. After natural transformation (Wang *et al.*, 1993) with the appropriate PCR product and selection on Cm-containing media, four to eight clones were evaluated by PCR to confirm replacement of the wild-type allele with the null allele; urease activity and flagella-based motility were also confirmed. Single clones were used for infection experiments and phenotypic characterization. J166 deletion mutants of *recA*, *addA*, and *ruvC* were constructed by allelic exchange using methods similar to those previously described (Akopyants *et al.*, 1998). Briefly, a non-polar kanamycin resistance (*aphA*) cassette (Menard *et al.*, 1993) and ~1 kb upstream and downstream "arms" for each gene were PCR amplified using primers (Table 3) with compatible 5' restriction sites. All three fragments were restricted with the appropriate enzymes, ligated with pBluescript SK(-) (Stratagene, La Jolla, CA) plasmid DNA which was previously digested with XhoI/NotI, and transformed into One Shot TOP10 competent *E. coli* (Invitrogen, Carlsbad, CA) with kanamycin selection. Each shuttle plasmid was verified by sequencing and transferred into *H. pylori* J166 using natural transformation with kanamycin selection, thereby deleting most of the coding region of each gene.

Generation of *E. coli* Expression Constructs and *H. pylori* Complementation Constructs

The *E. coli* expression construct pJF30 containing both AddA and AddB in pETDuet-1 (Novagen) was made by separately amplifying *addA* (*HP1553*) and *addB (HP1089*) from *H. pylori* strain 26695 using primers AddA-C1(*Sal*I), AddA-N1(*Nco*I), AddB N1 (*Nde*I), and AddB C1 (*Avr*II). The reaction conditions included six cycles with 54ºC annealing temperature followed by 24 cycles with 62ºC

460 annealing temperature and used High Fidelity Taq and Supermix (Invitrogen). Each gene was
461 separately cloned into pETDuet-1 after digestion of the PCR product and the vector with the indicated
462 restriction enzymes using standard procedures to generate pJF25 (*addA*), and pJF22 (*addB*). pJF30 was
463 made by subcloning *addB* from pJF22 into pJF25 using *Nde*I and *Avr*II. Vectors for complementation
464 were made by subcloning each gene individually into pRdxA and introduced into *H. pylori* NSH57 by
465 natural transformation and selection on Mtz-containing media (Smeets et al., 2000). The *addA*
466 complementing vector pJF29 was made by subcloning *addA* from pJF31 using *Xba*I and *Sal*I. The
467 *addB* complementing vector pJF27 was made by subcloning *addB* from pJF22 using *Avr*II and *Xba*I.
468 All inserted genes contained the expected nucleotide sequences, except for *addB* in pJF22, pJF30,
469 pJF31, and pJF27, which contained a single point mutation [T2311 → C2311] changing serine 771 to
470 proline (S771P). This residue is not part of any conserved domain of AddB, and the S771P-containing
471 clone fully complemented drug sensitivity and animal infectivity in *H. pylori* (Table 3 and Figure 4).
472 For assaying enzymatic activities, this mutation was repaired using QuikChange (Strategene) on the
473 pJF30 template to generate pSA405.
474

475 **Preparation of Cell-free Extracts and Enzymatic Assays**

476 Extracts were prepared as described by (Tomizawa and Ogawa, 1972). For *H. pylori* extracts bacteria
477 were harvested from 24 – 48 hour plate-grown cultures. For *E. coli* extracts bacteria were harvested 3
478 hr after addition of 1 mM IPTG to induce expression of AddA and AddB. ds exonuclease activity was
479 assayed as ATP-dependent solubilization of uniformly [^{3}H]-labeled T7 DNA (specific activity $2x10^4$
480 cpm/μg; Eichler and Lehman, 1977). Each assay included two or three protein concentrations that gave
481 a linear relationship between acid-solubilized DNA and protein assayed. Assays of haploid *H. pylori*
482 extracts contained 10 mM $MgCl_2$ and 1 mM ATP (see Figure S1). Assays of *H. pylori* AddAB in *E.*
483 *coli* extracts contained 50 μM ATP (Table 2).

The substrate for DNA unwinding was plasmid pBR322 digested with *Hin*dIII (New England Biolabs), treated with shrimp alkaline phosphatase (US Biochemicals), and labeled at the 5' ends with [γ-^{32}P] ATP (GE Biosciences). Unincorporated nucleotides were separated from the DNA substrate by passage through an SR200 minicolumn (GE Biosciences).

DNA unwinding assay mixtures contained 4.0 nM DNA substrate in 15 µl of buffer containing 25 mM Tris acetate (pH 7.5), 2 mM magnesium acetate, 5 mM ATP, 1 mM DTT and 1 µM single-stranded DNA binding protein (Promega). Reactions were for 2 min at 37^{o}C with the amount of extract protein indicated in Figure 1. Reactions were terminated by the addition of 5 µl of buffer containing 0.1 M EDTA, 2.5% SDS, 0.125% bromophenol blue, 0.125% xylene cyanol, and 10% ficoll. Reaction products were separated on a 0.7% agarose gel (22 cm long) in Tris acetate electrophoresis (TAE) buffer (Sambrook et al, 1989) at 100 V for 2 hr and visualized by autoradiography.

Efficiency of Plaque-formation by Phage T4 and T4 *2*$^{-}$

E. coli strain V3060 (chromosomal Δ*recBCD*) containing plasmids bearing the *E. coli recBCD* or *H. pylori addAB* genes was grown in TB broth containing ampicillin (100 ug/ml) to about 2 x 10^{8} cells/ml. Phage T4 or T4 *2*$^{-}$ in SM (50 µl) were added to 0.1 ml of bacteria and incubated for 15 min at 37^{o}C. Top agar (2.5 ml) was added and the mixture poured onto a TB agar plate. Plates were incubated at 37^{o}C overnight, after which plaques were enumerated.

Antibiotic Resistance Testing

Bacteria taken from fresh plates (incubated for 18 – 36 hr) were grown in liquid culture to an optical density at 600 nm (OD_{600}) between 0.1 and 1 and examined for spiral shape and motility. Five fold serial dilutions were spotted onto plates containing increasing concentrations of mitomycin C or

ciprofloxacin (Sigma). The minimal inhibitory concentration was determined when drug prevented growth of at least two five-fold serial dilutions.

UV Sensitivity

Bacteria were grown as described for antibiotic resistance testing. Dilutions were plated in duplicate, each on one half of a plate, and exposed to UV using a UV Stratalinker 2400 (Stratagene) on the energy setting. After UV exposure, plates were incubated for 3 – 4 days until single colonies could be counted. The percent survival was calculated in comparison to plates that were mock UV treated. The data presented are the average from three experiments.

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, autoclaved corn cob bedding, and acidified, reverse-osmosis purified water provided ad libitum. All studies were done under 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 FHCRC Institutional Animal Care and Use Committee.

For competition experiments each indicated null mutant strain and the parental wild-type strain or complemented mutant were grown from frozen stock in liquid culture to mid-to-late logarithmic growth phase. The wild-type and mutant bacteria were combined to give approximately 5.0×10^8 bacteria of each in 2.5 ml, and 0.5 ml inoculated by oral gavage into each of five mice. After inoculation, a portion of the inoculum was plated on HB-Bac plates and HB-Cm plates to determine the number of wild-type and mutant bacteria, respectively, in the inocula. After 1 week the mice were

euthanized by inhalation of CO_2. The glandular stomach was removed and cut along the greater curvature, and any food was removed. The stomach was then cut in half longitudinally, and one half stomach placed in 0.5 mL of BB10 broth for homogenization with disposable pellet pestles (Fisher Scientific). Dilutions of the homogenate were plated on HB-Bac to enumerate total bacteria and on HB-Cm plates to enumerate mutant bacteria. If no mutant bacteria were recovered we set the number of colonies on the lowest dilution plated to three. From the Poisson distribution we estimate with 95% confidence that a plate containing zero colonies corresponds to three or less colony forming units (CFU). The average bacterial colonization load for wild type in these experiments was 1.3×10^5 CFU/g and the range was $3.3 \times 10^3 - 9.6 \times 10^5$ CFU/g. The competitive index (CI) was computed as follows: CI = (output mutant CFU / output wild-type CFU) / (input mutant CFU / input wild-type CFU).).

To determine the dose for fifty percent infection (ID_{50}), the indicated strains were grown in liquid culture to mid-to-late logarithmic growth phase and concentrated to give $5 \times 10^9 - 5 \times 10^{10}$ bacteria per ml. Serial ten-fold dilutions were prepared and inoculated into each of five mice. After 7 – 10 days mice were euthanized and processed as described above; bacteria were enumerated on HB-Bac plates. The number of infected mice at each titer was determined (supplemental table S4), and the ID_{50} was calculated using the method of Reed and Muench (Reed and Muench, 1938).

babA Gene Conversion Assay

Real-time quantitative PCR was used to determine the frequency of apparent gene conversion of *babA* to *babB*, using methods similar to those described (Solnick et al., 2004). Briefly, genomic DNA was prepared from individual colonies of wild-type *H. pylori* J166, and from isogenic mutants with deletions of *recA*, *addA*, or *ruvC*. Each 20 µl reaction contained 10 µl of iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA), 5 µl of primer pair (1 µM each) containing a primer (Table S3) upstream of the *babA* locus (F14) and a primer in either *babA* (160R) or *babB* (178R), and 5 µl of

DNA template (0.0375 ng/µl for *babA*; 5 ng/µl for *babB*). Amplification was carried out in a Bio-Rad iCycler (3 min at 95°C; 45 cycles of 30 sec at 95°C, 30 sec at 58°C, 1 min at 72°C) and the cycle threshold (Ct) was determined. Standard curves were constructed for *babA* and *babB* using plasmids (Table S2) in which *babA* (pJ150) or *babB* (pJ151) was amplified and cloned into pGEM-T Easy (Promega, Madision, WI) using TA cloning. Template for amplification of the *babA* and *babB* fragments was chromosomal DNA from wild-type *H. pylori* J166 (*babA*) or *H. pylori* J166 passaged through rhesus macaques (*babB*), in which the *babA* gene was converted by *babB*. Primers for amplification of *babA* and *babB* (Table S3) were therefore the same, since the upstream primer (HP0898F) was not in *babA/babB* and the downstream primer (AN5954) was in a region in which *babA* and *babB* are identical.

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766
767

Figure Legends

Figure 1. Epsilon Proteobacteria AddA and AddB homologs with conserved nuclease domains. Nuclease domains of AddA (A) and AddB (B) proteins from epsilon Proteobacteria with fully sequenced genomes show highly conserved residues indicated by shading. Sites where mutations in *B. subtilis addA* and *addB* abolish nuclease activity (Kooistra et al., 1997) are indicated by an asterisk. Species names, and first and last residue numbers in the displayed alignment are shown on the left. Alignment to the nuclease domains of *E. coli* RecB and *B. subtilis* AddA (A) and *E. coli* RecB and *B. subtilis* AddB (B) are shown for comparison (bottom of each alignment). The corresponding locus tags and (GI numbers) for the epsilon Proteobacterial AddA proteins are (top to bottom) CCV52592_0910 (154174808), CFF8240_0386 (118474774), CHAB381_1391 (154148488), CJE1654 (57238504), JJD26997_1828 (153951355), CJJ81176_1474 (121612796), Cj1481c (15792796), CLA0743 (57240914), CUP1844 (57242577), Hac_0287 (109946901), HH1643 (32267142), HP1553 (15646160), HPAG1_1502 (108563927), jhp1446 (15612511), NIS_0366 (152990115), SUN_0187 (152991783), Tmden_0090 (78776291), WS1252 (34557616). The corresponding locus tags and GI numbers for the epsilon Proteobacterial AddB proteins are (top to bottom) Ccur5_02000280 (145956769), CFF8240_0385 (118475773), CHAB381_1392 (154149234), CJE1655 (57238505), Cjejd_02001773 (145960086), CJJ81176_1475 (121613506), Cj1482c (15792797), CLA0742 (57240913), CUP1843 (57242576), Hac_0793 (109947353), HH0025 (=(32265524), HP1089 (15645703), HPAG1_0358 (108562783), jhp0336 (15611404), NIS_0365 (152990114), SUN_0185 (152991781), Tmden_0088 (78776289), WS0562 (34556981).

Figure 2. AddAB promotes ATP-dependent DNA unwinding. Extracts were prepared from strain V3060 (Δ*recBCD2731* DE3) carrying vector pETDuet-1 with or without insertion of the *H. pylori addA* and *addB* genes or pBR322 with *recBCD* (pMR3). The indicated amount of extract protein was

791 incubated with pBR322 linearized and 5' end-labeled with ^{32}P. Reactions contained ATP (5 mM) as
792 indicated. The positions of ds DNA substrate (ds), unwound ss DNA (ss) produced by boiling or
793 enzymatic reaction, and degraded DNA (nuc) are shown.
794
795 Figure 3. AddAB confers modest protection against UV damage. The indicated bacterial strains (wild-
796 type, mutant, and complemented mutant strains) were exposed to UV irradiation, and the fraction of
797 bacteria surviving was determined. The data plotted represent averages of three separate experiments,
798 and bars indicate 1 standard deviation.
799
800 Figure 4. AddAB and RecA promote stomach colonization. Mice were orally infected with a 1:1
801 mixture of the indicated strains. The actual ratio in the inoculum of mutant to wildtype or
802 complemented mutants is indicated. After one week the bacteria colonizing the stomach were
803 harvested and the competitive index determined (ratio of mutant to wild type in the output corrected
804 for the input ratio) as described in the Experimental Procedures. Each data point is from one mouse,
805 and the geometric means are indicated by horizontal bars. Infections with two independent *ΔrecA::cat*
806 clones are indicated by open and filled circles. Bracketed data points represented an upper limit on the
807 competitive index with 95% confidence because of failure to isolate mutant clones from the stomach as
808 described in the Experimental Procedures. Competitions of mutant clones with wild type were repeated
809 with similar results (data not shown). A colonization defect is indicated by a competitive index of less
810 than one.
811
812 Figure 5. AddA and RecA promote *babA* to *babB* gene conversion. Quantitative PCR was used to
813 determine the number of copies of *babA* and *babB* at the *babA* locus for genomic DNA prepared from
814 each of three cultures started from individual colonies as described in the Experimental Procedures.
815 The geometric mean of the frequency of *babB*/*babA* at the *babA* locus is reported and the bars indicate

816 1 standard deviation. The difference in means between groups was considered significant (one-way
817 analysis of variance, $p = 0.0002$). Significant P values for pair-wise comparisons between groups are
818 indicated $^{**}p < 0.001$, $^{*}p < 0.01$.

Table 1. *H. pylori addA* and *addB* Mutants Lack ATP-dependent ds DNA Exonuclease Activity

Relevant genotype[a]	Allele at *rdx*[b]	ATP-dependent ds DNA exonuclease (Units/mg extract protein)	
		Mean[c]	Range
WT	-	5.3 ± 0.9 (6)	3.4 – 6.8
ΔaddA::cat	-	0.009 ± 0.0007 (6)	0.009 – 0.010
ΔaddA::cat	*addA*$^+$	6.0 (2)	5.7 – 6.3
ΔaddB::cat	-	0.02 ± 0.017 (6)	0.010 – 0.040
ΔaddB::cat	*addB*$^+$	7.1 (2)	7.0 – 7.2
ΔrecA::cat	-	6.1 ± 0.25 (3)	5.9 – 6.4
ΔruvC::cat	-	4.8 ± 0.4 (3)	4.3 – 5.1

[a]These strains are derivatives of *H. pylori* strain NSH57 with the indicated deletion allele on the chromosome.

[b]The indicated allele is at the *rdx* chromosomal locus. "-" indicates wild-type *rdx*.

[c]The values are the means and standard deviations of the number of extracts assayed in parentheses. For those experiments where two extracts were assayed only the mean is indicated.

Table 2. Cloned *H. pylori addA* and *addB* Genes Express ATP-dependent Exonuclease in *E. coli* Cells

Plasmid	ATP-dependent ds DNA exonuclease (Units/mg extract protein) [a]	Efficiency of plating[b]	
		T4	T4 *2*$^{-}$
pBR322	18 ± 4 (4)	1.0	1.0
pMR3 (*recBCD*)	140 ± 11 (6)	0.94	8.3×10^{-7}
pETDuet-1	24 ± 5 (6)	0.97	0.91 ± 0.04
pSA405 (*addA addB*)	880 ± 12(6)	0.91	6.2×10^{-6}

[a]Extracts were prepared from transformants of *E. coli* strain V2831 (Δ*recBCD2731* <*kan*>) with pBR322 or pMR3 or transformants of *E. coli* strain V3060 (Δ*recBCD2731* <*kan*> DE3) with pETDuet-1 or its derivatives. Cells containing pETDuet-1 were harvested 3 hr after induction with 1 mM IPTG. Data are the mean ± SEM from the indicated number of extracts (n) from separate cultures.

[b]Phage titer on *E. coli* strain V3060 (Δ*recBCD2731* <*kan*> DE3) with the indicated plasmid divided by the phage titer on strain V3060 with pBR322. Data are the mean from 3 separate experiments.

Table 3. Antibiotic Sensitivity of Recombination Mutants

Genotype[a]	Minimal inhibitory concentration	
	Mitomycin C (ng/ml)	Ciprofloxacin (μg/ml)
WT	6.0	0.5
ΔrecA::cat	1.8	0.1
ΔaddA::cat	1.8	0.1
ΔaddB::cat	1.8	0.1
ΔaddA::cat, rdxA::addA	6.0	0.5
ΔaddB::cat rdxA::addB	6.0	0.5

[a]NSH57 strain background.

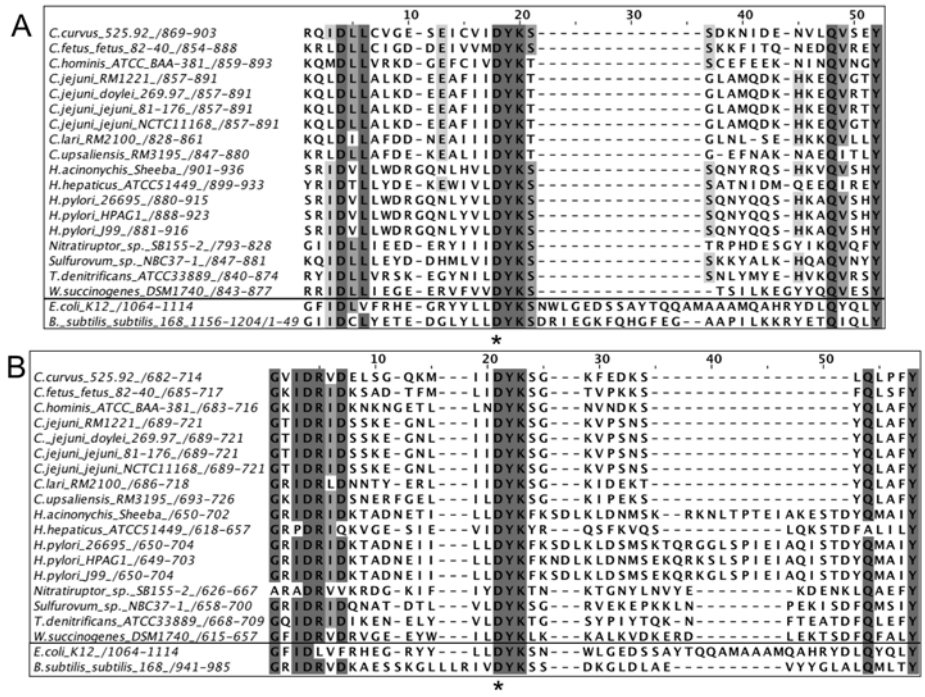

Figure1

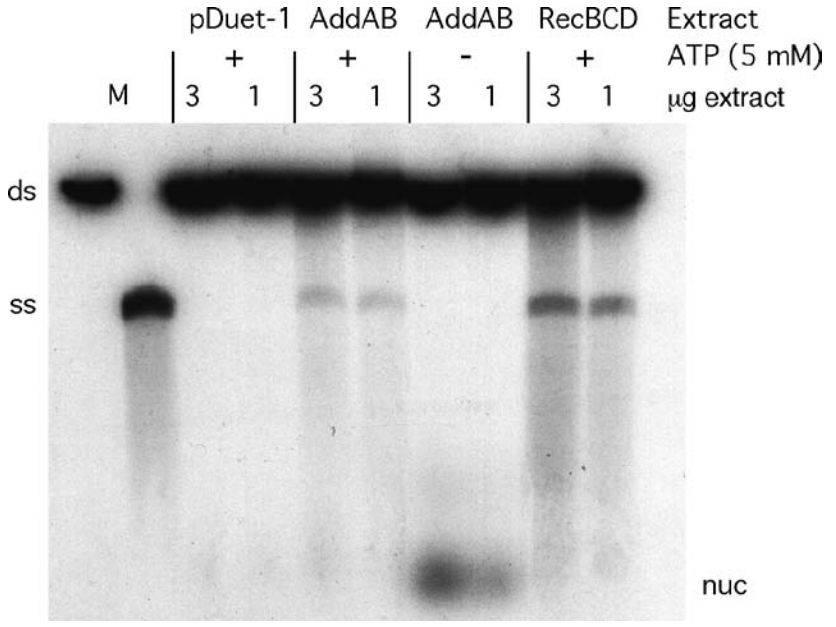

Figure2

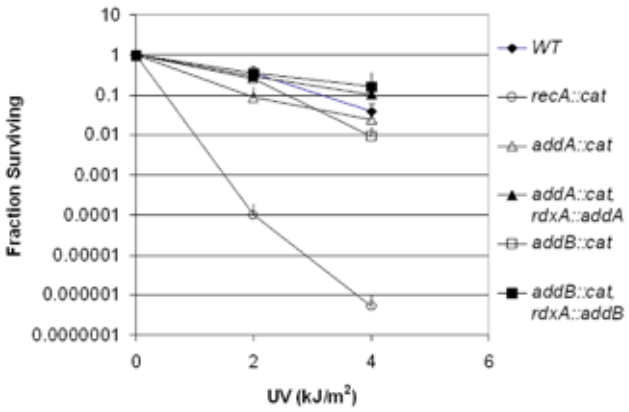

Figure3

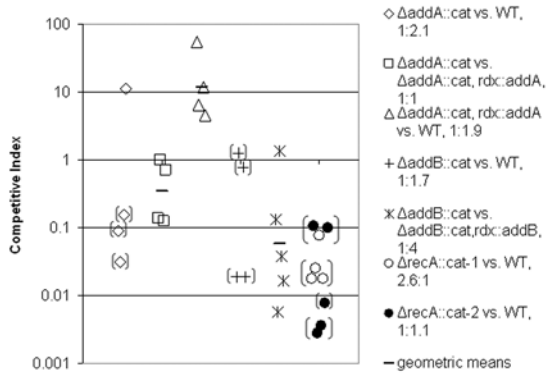

Figure4

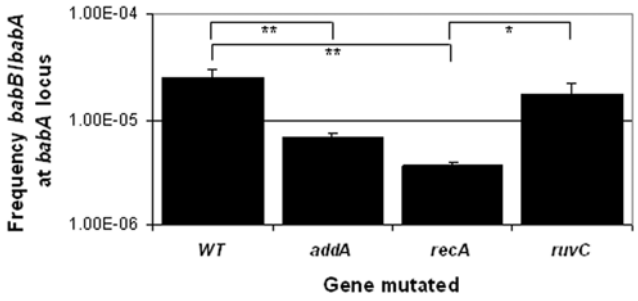

Figure5