INFECTION AND IMMUNITY, June 1995, p. 2185–2193 Vol. 63, No. 6
0019-9567/95/$04.00+0

Isolation of the *Helicobacter pylori recA* Gene and Involvement of the *recA* Region in Resistance to Low pH

STUART A. THOMPSON* AND MARTIN J. BLASER

Division of Infectious Diseases, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Received 9 January 1995/Returned for modification 23 February 1995/Accepted 7 March 1995

To understand the potential roles of the important DNA repair protein RecA in *Helicobacter pylori* pathogenesis, we cloned the *recA* gene from *H. pylori* 84-183. Degenerate PCR primers based on conserved RecA protein regions were used to amplify a portion of *H. pylori recA*, which was used as a probe to isolate the full-length *recA* gene from *H. pylori* genomic libraries. The *H. pylori recA* gene encoded a protein of 347 amino acids with a molecular mass of 37.6 kDa. As expected, *H. pylori* RecA was highly similar to other RecA proteins and most closely resembled that of *Campylobacter jejuni* (75% identity). Immediately downstream of *recA* was an open reading frame whose predicted product showed 58% identity to the *Bacillus subtilis* enolase protein. *recA* and *eno* were disrupted in *H. pylori* 84-183 by insertion of antibiotic resistance genes. Reverse transcription-PCR demonstrated that *recA* and *eno* were cotranscribed and that insertion of the kanamycin resistance gene into *recA* had polar effects on expression of the downstream *eno*. The *H. pylori recA* mutants were severely impaired in their ability to survive treatment with UV light and methyl methanesulfonate and with the antimicrobial agents ciprofloxacin and metronidazole. The *eno* mutant had sensitivities to UV light and metronidazole intermediate to those of wild-type and *recA* strains, suggesting that truncation of the *recA-eno* transcript resulted in lowered *recA* expression. For survival at low pH, a *recA* mutant was approximately 10-fold more sensitive than strain 84-183, while the *eno* mutant demonstrated intermediate susceptibility. This difference occurred in the presence or absence of urea, implying the involvement of a gene in the *recA* region in an acid resistance mechanism distinct from that mediated by urease.

Helicobacter pylori is a microaerophilic, gram-negative bacterium that causes chronic gastritis in humans, which may lead to peptic ulceration or gastric carcinoma (4). It is therefore important to understand the factors involved in the pathogenesis of *H. pylori*. Some of the previously described virulence factors of *H. pylori* include flagella (14, 40), cytotoxin (7, 39), the cytotoxin-associated CagA protein (67), and urease (46). Although *H. pylori* strains are similar phenotypically (with the exception of variability in cytotoxin and CagA production) (7, 50, 67), they exhibit a high degree of restriction site polymorphism among strains (1, 6, 17, 18, 41).

On the basis of functions studied in *Escherichia coli* and other bacteria studied (70), we chose to investigate the presence in *H. pylori* of the important recombination protein RecA. Since RecA is essential for DNA recombination, *recA* mutants may be valuable as attenuated vaccine strains (22). The virulence of *recA* mutants may be diminished (5), yet such strains may still be able to induce a significant immune response (25). If used in conjunction with other attenuating mutations, the lack of RecA function in a vaccine strain may stabilize these mutations and reduce or eliminate the possibility of reversion to virulence. In addition, most *H. pylori* strains are naturally competent for DNA uptake (71). It is likely that RecA-mediated recombination is necessary for DNA transformation (25, 33, 51, 69); it is possible that this might play a role in genomic variability through uptake of DNA from neighboring cells.

In addition to its role in recombination, in many bacterial genera, RecA is a regulatory protein that responds to certain types of DNA damage and mediates derepression of a family of proteins, called the SOS regulon (70). The net effects of SOS induction are many and include increased DNA repair, an increased rate of mutation, inhibition of cell division, and prophage induction (70). The SOS response is important in the repair of DNA damage caused by exposure to reactive oxygen species produced by immune cells during bacterial infection (21, 26, 29). Such a response may therefore be important for survival of *H. pylori* during infection of the gastric mucosa, during which a high level of inflammation and neutrophil infiltration occurs (68).

We now report the identification and cloning of *recA* in *H. pylori*. Mutation of *recA* affects the survival of *H. pylori* cells after exposure to a variety of noxious stimuli, including low pH.

MATERIALS AND METHODS

Strains and growth conditions. Strains and plasmids used in this study are listed in Table 1. *E. coli* was routinely grown in Luria-Bertani (LB) broth or agar (55) supplemented with carbenicillin (100 μg/ml), kanamycin (30 μg/ml), and/or chloramphenicol (30 μg/ml) when appropriate. *H. pylori* strains were grown on blood agar plates (BAP) at 37°C in a 5% CO_2 atmosphere. Antibiotic-resistant *H. pylori* strains were selected with 30 μg of kanamycin per ml or 15 μg of chloramphenicol per ml.

DNA techniques. Restriction enzymes and Klenow fragment of *E. coli* DNA polymerase I were purchased from either New England Biolabs (Beverly, Mass.) or Promega (Madison, Wis.) and used as described in the manufacturer's directions. *H. pylori* chromosomal DNA was prepared as described previously (57). Hybridizations with ^{32}P-labeled probes labeled by random priming (15) with a kit from Boehringer-Mannheim Biochemical Corp. (Indianapolis, Ind.) were done as described previously (3). DNA sequencing was done either on double-stranded templates (34) by the method of Sanger et al. (56) with a Sequenase 2.0 kit (U.S. Biochemical Corp., Cleveland, Ohio) or on an ABI automated sequencer by the Vanderbilt University Cancer Center Core Facility. The DNA sequence resulting from unambiguous reading of both strands was compiled with the Staden alignment programs (61). Computer analyses of DNA and protein sequences were performed with the Genetics Computer Group programs (10), and database similarity searches were performed via electronic mail to the National Center for Biotechnology Information by use of the BLASTX algorithm (2, 20).

PCR amplification and cloning of *H. pylori recA*. Degenerate PCR primers were designed on the basis of conserved amino acid sequences of RecA and

* Corresponding author. Mailing address: Division of Infectious Diseases, Vanderbilt University School of Medicine, MCN A-3310, Nashville, TN 37232. Phone: (615) 322-2035. Fax: (615) 343-6160. Electronic mail address: thompssa@ctrvax.vanderbilt.edu.

TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Relevant genotype	Reference
E. coli		
Y1089	*recA*$^+$	75
DH5αMCR	*recA1*	27
H. pylori		
84-183	*recA*$^+$	50
93-225	*recA*$^+$ *cagA*::*km*	This study
93-226	*recA*::*km*	This study
93-227	*recA*::*cm*	This study
94-49	*eno*::*km*	This study
Plasmids		
pSAT101	*recA* (5′ end)	This study
pSAT104	*recA* (3′ end)	This study
pSAT105	*recA* (reconstructed)	This study

synthesized at the Vanderbilt University Diabetes DNA Core Facility in a Milligen 7500 DNA synthesizer. Primer Rec1F (5′ GARATHTWYGGNCCNGA 3′) was based on the amino acid sequence EIYGPE. Primer Rec2R (5′ GCRT ARAAYTTNARNGC 3′) was based on the reverse complement of the amino acid sequence NALKFYA. Thermocycler parameters were as follows: 1 min at 94°C (denaturation); 2-min ramp to 37°C, followed by 2 min at 37°C (primer annealing); 2-min ramp to 60°C, followed by 2 min at 60°C (extension). The degenerate PCR product was subcloned into the pT7Blue(R) T-vector from Novagen (Madison, Wis.) by ligating the vector and insert at a molar ratio of 3:1 (vector/insert). λZAPII (Stratagene, La Jolla, Calif.) was used to prepare two genomic libraries from *H. pylori* 84-183. For the first, chromosomal DNA from strain 84-183 was partially digested with *Alu*I, and 2- to 7-kb fragments were purified and ligated with *Eco*RI linkers. The second library was constructed from chromosomal DNA digested to completion with *Hin*dIII, end-repaired by Klenow enzyme, and ligated with *Eco*RI linkers. Both types of fragments were ligated separately with *Eco*RI-digested, alkaline phosphatase-treated λZAPII arms and packaged into bacteriophage λ heads with packaging extracts (Stratagene or Promega). Following identification of the desired λ clones by hybridization with the PCR-amplified fragment of *H. pylori recA*, plaques were purified and recombinant pBluescript plasmids were recovered following the addition of R408 helper phage.

Mutagenesis of cloned *H. pylori recA* and *eno* genes. Fragments containing the genes for resistance to kanamycin (Km^r) and chloramphenicol (Cm^r) were prepared as follows. The Km^r gene was purified from *Sma*I-digested pILL600 (37). The Cm^r gene from pRY109 (74) was subcloned into the *Pst*I site of pBluescript to create pBSC103 (12a) and then isolated following *Hin*cII and *Sma*I digestion. The *recA*-containing plasmid pSAT101 was linearized at a unique *Sty*I site located within codon 31 of the *recA* open reading frame (ORF). After filling these ends with Klenow enzyme, the molecule was ligated to either the Km^r or Cm^r fragments. After transformation into *E. coli* DH5αMCR and selection on appropriate antibiotics (carbenicillin and kanamycin or carbenicillin and chloramphenicol), a single colony was chosen from each ligation (pSAT101::*km* and pSAT101::*cm*). To mutagenize the *eno* gene, pSAT104 was linearized at the unique *Age*I site in codon 44 of the *eno* gene. The ends of this fragment were made blunt with Klenow enzyme and ligated with the blunt-ended Km^r fragment as described above. A plasmid that contained the desired insertion was isolated and designated pSAT104::*km*. The orientations of Km^r and Cm^r fragment insertions in each of the constructs described above were determined by DNA sequencing.

Electroporation and natural transformation of *H. pylori* 84-183. For electroporation, *H. pylori* 84-183 cells were scraped from 24-h cultures on four BAP and washed as described previously (58). Approximately 1 μg of supercoiled donor plasmid was mixed with the washed cells, placed in a Gene-Pulser (Bio-Rad, Melville, N.Y.), and electroporated at 2500 V, 200 W, and 25 μF (58). Cells were then inoculated onto BAP without antibiotics. Following overnight growth at 37°C, the entire contents of the plate were swabbed and streaked onto BAP containing either kanamycin or chloramphenicol. Antibiotic-resistant colonies were harvested after 3 days of growth at 37°C. Electroporations with either no donor DNA or the nonmutagenized pSAT101 served as negative controls. Natural transformation experiments were done essentially as described previously (71) by concentrating the cells from a 24-h culture grown on one BAP into 50 μl of saline. Following the application of 10-μl spots of concentrated cells to a BAP, 10 μl of saline containing pSAT104::*km* (1 μg) or saline alone (negative control) was added to the cells, and the mixture was incubated at 37°C overnight. The following morning, the cells were streaked onto BAP containing kanamycin. Kanamycin-resistant colonies were recovered as described above.

Sensitivities of mutants to UV light, MMS, and antimicrobial agents. Assays to determine the sensitivities of *H. pylori* strains to UV radiation and methyl methanesulfonate (MMS) were performed as follows. Two hundred to 400 CFU of each strain (from 48-h BAP cultures) were plated onto either BAP (UV experiments) or BAP containing various concentrations of MMS (Kodak Co., Rochester, N.Y.) to achieve a countable range of surviving colonies from a single plate. For UV experiments, the plates were dried briefly and exposed for various amounts of time to 254-nm UV light generated by a Universal UV lamp (model 51402; Gelman-Camag) at a distance of 10.5 cm. The exposures were performed in a photographic darkroom, and the plates were immediately wrapped in aluminum foil to prevent photoreactivation. Plates from both types of experiments were incubated for 5 days before the colonies were counted and the survival was determined. Determinations (in triplicate) of the MICs for the antimicrobial agents ampicillin, ciprofloxacin, erythromycin, metronidazole, and tetracycline were achieved by the E-test (Remel Corp., Lenexa, Kans.), in which E-strips were placed onto BAP inoculated with 0.5 McFarland standard suspensions of 48-h cultures of *H. pylori* strains. MICs were scored after 3 days of growth.

Sensitivity of mutants to low pH. With the assay of McGowan et al. (43), approximately 10^8 CFU of the wild-type strain 84-183, the *recA* mutant 93-226, or the *eno* mutant 94-49 were incubated in phosphate buffer (pH 7.0, 4.0, or 3.3), in either the presence or absence of 10 mM urea. Following a 1-h incubation at 37°C, the cells were diluted in phosphate buffer (pH 7.0) and rapidly spread on BAP. The number of surviving colonies was assayed following 5 days of growth. We determined the number ($\log_{10}$) of bacteria killed at pH 4.0 or 3.3 compared with that occurring at pH 7.0. The statistical significance was determined with a one-tailed Student's *t* test.

Reverse transcription (RT)-PCR. RNA was prepared from 30-h cultures of wild-type and mutant *H. pylori* strains grown on BAP as described previously (55). A 10-μg sample of each RNA was treated with RNase-free DNase (Promega) to remove contaminating DNA. We next prepared cDNA from 1-μg aliquots of the DNase-treated RNA samples as described previously (48), by use of Moloney murine leukemia virus reverse transcriptase (Gibco-BRL, Gaithersburg, Md.) and random hexamer oligonucleotide primers (Pharmacia LKB Biotech, Piscataway, N.J.). The reaction mixtures were diluted to 20 μl with H_2O, and 1 μl of each was used as a template for PCR (48) with oligonucleotide primers specific for various regions of the *recA*, *eno*, and Km^r genes (Table 2). PCR products were analyzed by electrophoresis through 1.0% agarose.

The sequence reported in Fig. 2 has been deposited in GenBank under accession number U13756.

TABLE 2. PCR primers used in this study

PCR primer	Amino acid sequence or positions[a]	Nucleotide sequence
Degenerate		
Rec1F	EI(Y/F)GPE	5′ GARATHTWYGGNCCNGA 3′
Rec2R	NALKFYA	5′ GCRTARAAYTTNARNGC 3′
Nondegenerate		
Rec101	539–558	5′ GAAATTTATGGGCAGAGTC 3′
Rec102	908–928 (complement)	5′ GATAAAAATGAGAGTGGTGTT 3′
Rec112	1052–1071	5′ CAAAACGAACAGCATATCGG 3′
Eno101	1631–1651	5′ AACACCATTTAATAGGGCTTG 3′
Eno104	1479–1500 (complement)	5′ CTTAGTGTTATCGCTTAAAATC 3′
Eno-R	1912–1933 (complement)	5′ CTTTAAAACTCTCAAACCCTAA 3′
km-F		5′ TGTAGAAAAGAGGAAGGAAA 3′
km-R		5′ CTAAAACAATTCATCCAGTA 3′

[a] See Fig. 2.

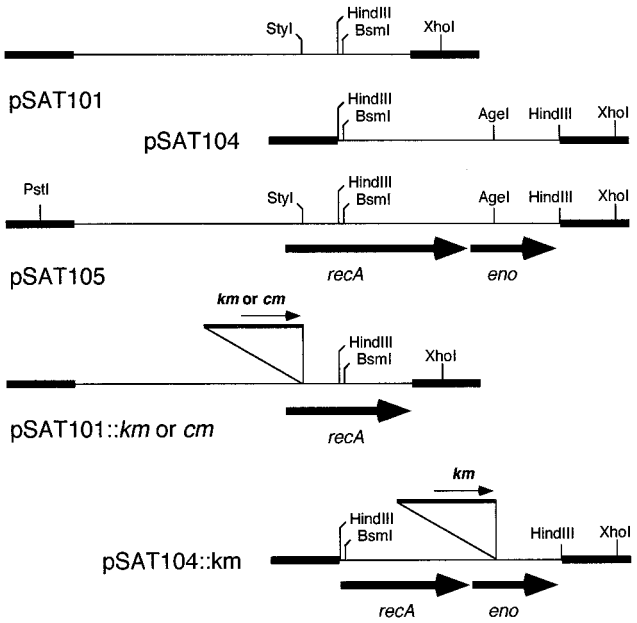

FIG. 1. Clones isolated in this study. The *recA* gene was reconstructed in *E. coli* by ligating the *Bsm*I-*Xho*I fragment of pSAT101 (containing the 5′ end of *recA*) with the *Bsm*I-*Xho*I fragment of pSAT104 (containing the 3′ end of *recA*). Genes for resistance to kanamycin (*km*) or chloramphenicol (*cm*) were inserted into the unique *Sty*I site located within the *recA* ORF to create *recA* mutants. An *eno* mutant was created by insertion of *km* into the unique *Age*I site within the *eno* ORF.

RESULTS

PCR amplification of a portion of the *H. pylori recA* gene. To amplify a portion of the *H. pylori recA* gene by PCR, degenerate primers were developed on the basis of an alignment of known RecA protein sequences involving the highly conserved peptides EIYGPE and NALKFYA (54). Degenerate PCR of *H. pylori* 84-183 chromosomal DNA using these primers resulted in the amplification of four products, ranging in size from 350 to 800 bp. One of these was approximately the expected size (470 bp) and was subsequently subcloned into pT7Blue. A BLAST search of GenBank with the sequence of the subcloned PCR product verified that it was a portion of the *H. pylori recA* gene. To isolate the entire *recA* gene, we screened a λZAPII library constructed previously from partially *Alu*I-digested *H. pylori* 84-183 chromosomal DNA. The PCR-amplified *recA* fragment was used as a probe and identified three plaques containing *recA* sequences. Each recombinant was excised to a pBluescript plasmid by the addition of helper phage. Restriction analysis of these clones revealed that all contained identical 2.3-kb inserts (data not shown). One of these was designated pSAT101 (Fig. 1) and was subjected to DNA sequence analysis. One end of the insert was within an 820-bp ORF, whose predicted amino acid sequence showed high similarity to bacterial RecA proteins when used in a BLAST search of the GenBank data library. Since the 3′ end of this ORF was not contained on pSAT101, we constructed a library of strain 84-183 DNA digested to completion with *Hin*dIII. With the *recA* PCR probe, we identified a clone which contained the desired 1.4-kb *Hin*dIII fragment. The resulting excised plasmid (pSAT104) was found to contain the 3′ end of the *recA* gene as well as a portion of an ORF immediately downstream (Fig. 1). The intact *recA* gene was reconstructed from pSAT101 and pSAT104 as follows. A 3.8-kb *Bsm*I-*Xho*I fragment of pSAT101 containing the vector and the 5′ end of *recA* was ligated with the 1.4-kb *Bsm*I-*Xho*I fragment of pSAT104 (containing the 3′ end of *recA*). The desired plasmid (pSAT105) was isolated following transformation of *E. coli* DH5αMCR (Fig. 1). The DNA sequences of the ligation junctions were determined to verify correct assembly. A search of the GenBank data library identified a high similarity between the protein predicted by the downstream partial ORF and enolase proteins (Eno) of bacteria, yeasts, and mammals. Similarities to bacterial enolases were approximately 73 to 74% (*Bacillus subtilis* and *Zymomonas mobilis*). The location of the *eno* gene immediately downstream from *recA* was similar to the arrangement at the *recA* locus in *Campylobacter jejuni* (25).

Features of *H. pylori recA* and *eno* sequences. The adjoined DNA sequences of pSAT101 and pSAT104 (Fig. 2) identify the presence of two genes. The 1,044-bp *recA* ORF begins with the ATG at position 350 and is preceded by a putative (AGGT) ribosome binding site located at position 340. The *recA* gene terminated at position 1393 with a TAA codon. Immediately following the *recA* termination codon was the probable ribosome binding site (AGGA) for the *eno* gene at position 1396. The ATG codon at position 1405 initiated a 533-bp ORF (*eno*), which continued to the end of the DNA cloned in pSAT104. The only consensus promoter elements in this region were −35 and −10 sites preceding the *recA* ORF, at positions 264 (TTGTGA) and 288 (TATAAT), respectively. No transcriptional terminator was present between the *recA* and *eno* genes, although an 11-bp inverted repeat resembling a terminator ($\Delta G = -7.5$ kcal/mol [ca. −31 kJ/mol]) was found within the *eno* gene at positions 1570 to 1598 (Fig. 2). The protein predicted by the *recA* ORF was 347 amino acids in length and had a molecular mass of 37.6 kDa. As expected because of the high degree of amino acid conservation between bacterial RecA proteins (54), the predicted protein sequence of *H. pylori* RecA shared several features with 44 known RecA sequences. The amino acid residues that are highly conserved or invariant in bacterial RecA proteins and related bacteriophage and yeast recombination proteins identified by Story et al. (62) were all present and in the predicted locations in the deduced amino acid sequence of the *recA* ORF (Fig. 2). The residues identified were glycine 67, lysine 73, threonine 74, aspartic acid 95, glutamic acid 97, tyrosine 104, aspartic acid 145, serine 146, asparagine 194, glutamine 195, and glycine 213, and these loci are known to be involved in ATP binding, DNA interactions, or stabilization of RecA protein structure (62). The overall homology of *H. pylori* RecA to other bacterial RecA proteins was between 75% (*C. jejuni*) and 54% (*Acidiphilium facilis*) amino acid identity. It is interesting that *H. pylori* RecA is most closely related to *C. jejuni* RecA, since *H. pylori* was classified previously as a member of the genus *Campylobacter*.

Construction of *recA* and *eno H. pylori* strains. To construct *recA* mutants of *H. pylori* 84-183, we inserted either a Km^r gene (37) or a Cm^r gene (74) into the unique *Sty*I site of pSAT101 (within codon 31 of the *H. pylori recA* gene) (Fig. 2). These insertions would result in premature termination of *recA* translation either 56 bp (Km^r fragment) or 62 bp (Cm^r fragment) into the mutagenic cassettes. The resulting plasmids pSAT101::*km* and pSAT101::*cm*, respectively, were used as donor DNAs in electroporation of *H. pylori* 84-183. Following electroporation and plating on selective medium, we isolated 10 kanamycin-resistant colonies and one chloramphenicol-resistant colony. One kanamycin-resistant colony (strain 93-226) and one chloramphenicol-resistant colony (strain 93-227) were characterized further by Southern hybridization with probes for the *recA*, Km^r, or Cm^r genes and the pBluescript vector (Fig. 3). As

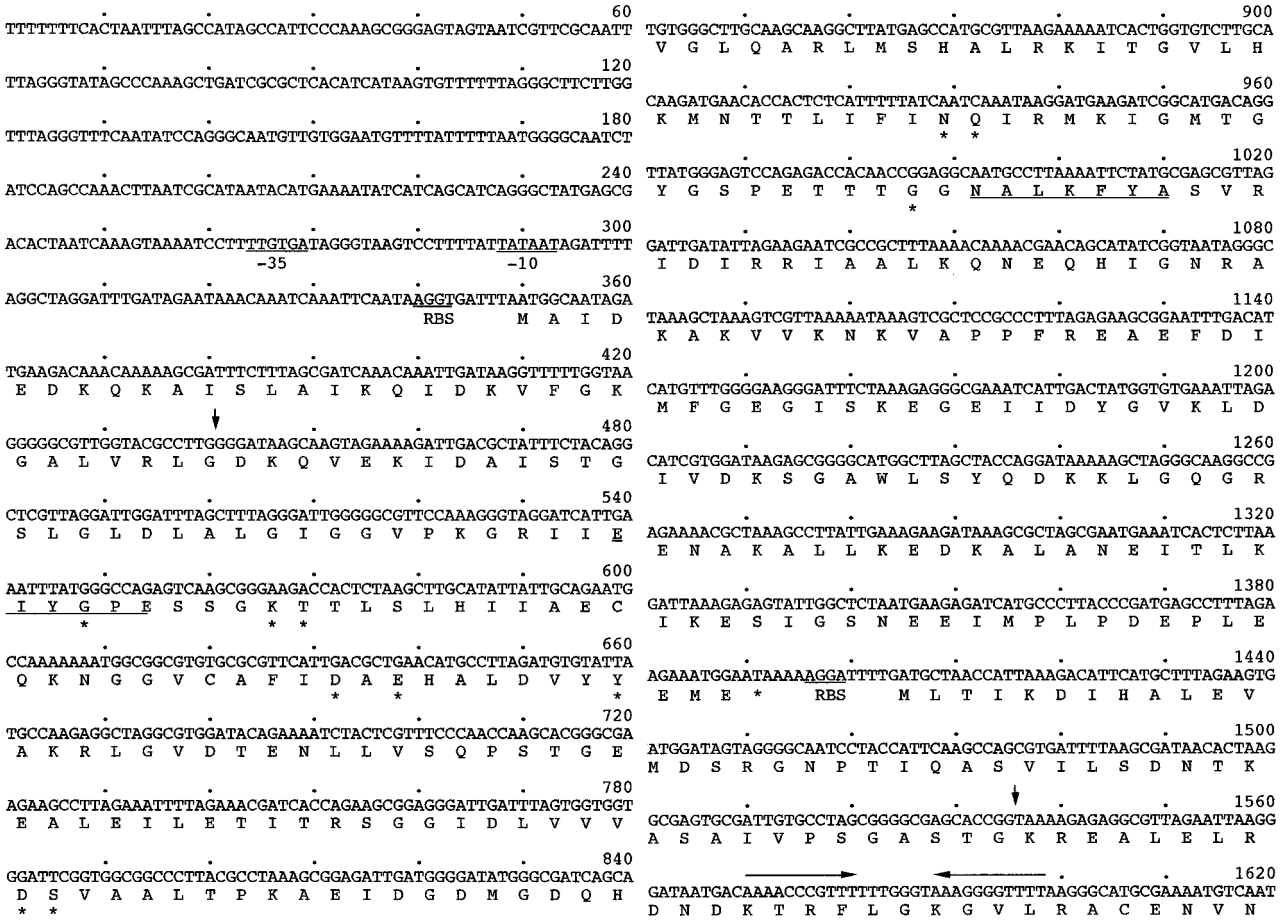

FIG. 2. Sequence of the *H. pylori recA-eno* region. Putative promoter and ribosome binding sites (RBS) are indicated. The *Sty*I site in codon 31 of the *recA* ORF and the *Age*I site in codon 44 of the *eno* ORF into which the Km^r or Cm^r resistance genes were inserted are shown as vertical arrows. The conserved RecA amino acid sequences on which the degenerate PCR primers were based are underlined. Amino acid residues which are highly conserved in RecA proteins (62) are indicated by asterisks. The inverted repeat within *eno* which may act as a transcriptional terminator is indicated by horizontal arrows.

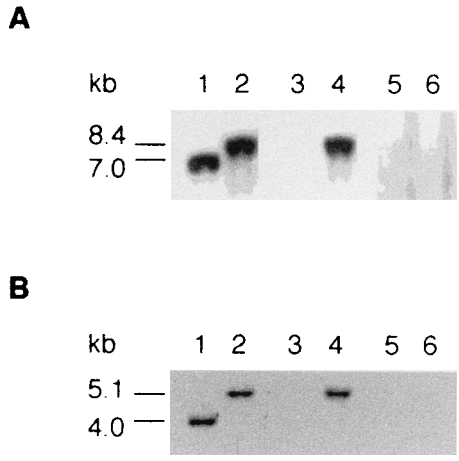

FIG. 3. Southern hybridization analysis of *H. pylori recA* mutants. Chromosomal DNA samples are from strains 84-183 (A and B, lanes 1, 3, and 5), 93-226 (A, lanes 2, 4, and 6), and 93-227 (B, lanes 2, 4, and 6), digested with *Sac*I (A) or *Ssp*I (B). Filters were probed with either the *recA* PCR fragment (A and B, lanes 1 and 2), the fragment of pILL600 encoding Km^r (A, lanes 3 and 4), the fragment of pRY109 encoding Cm^r (B, lanes 3 and 4), or pBluescript (A and B, lanes 5 and 6).

expected, insertion of the Km^r gene in strain 93-226 caused a 1.4-kb increase in the size of the 7.0-kb *recA*-containing *Sac*I restriction fragment (Fig. 3A, lanes 1 to 4). Similarly, insertion of the Cm^r gene caused a 1.1-kb increase in the 4.0-kb *recA*-containing *Ssp*I fragment of strain 93-227 (Fig. 3B, lanes 1 to 4). The lack of hybridization to pBluescript demonstrated that in neither strain was the insertion of the antibiotic resistance gene accompanied by insertion of vector sequences (Fig. 3, lanes 5 and 6). Southern analysis of the remaining nine kanamycin-resistant strains showed that they were identical to strain 93-226 (data not shown). To mutagenize the *eno* gene in strain 84-183, we inserted a Km^r gene into a unique *Age*I site

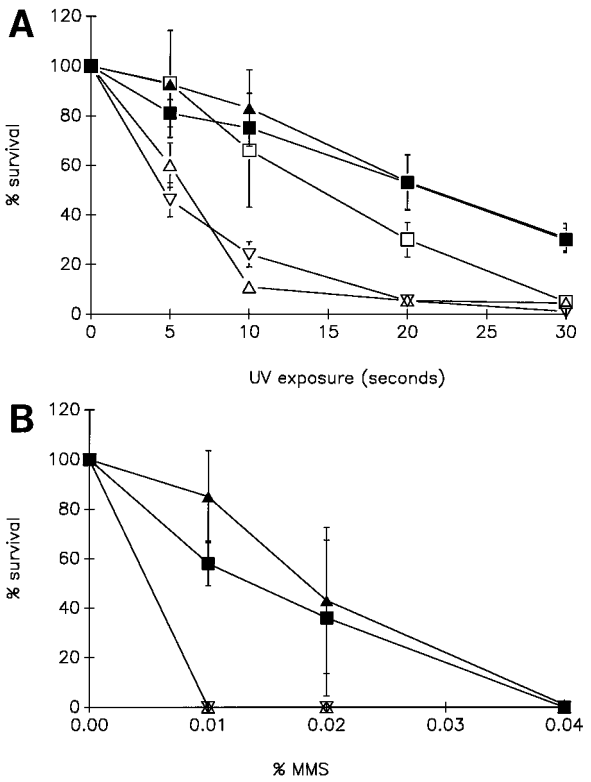

FIG. 4. Sensitivities of wild-type and mutant *H. pylori* strains to either UV radiation (A) or MMS (B) determined by plotting the fractional survival of each strain versus exposure time or dose of DNA damaging agent. Symbols: ▲, *H. pylori* 84-183 (*recA*$^+$); ■, *H. pylori* 93-225 (*recA*$^+$); △, *H. pylori* 93-226 (*recA*::*km*); ▽, *H. pylori* 93-227 (*recA*::*cm*); □, *H. pylori* 94-49 (*eno*::*km*).

in codon 44 of the *eno* ORF to yield pSAT104::*km*. The mutated *eno* gene was returned to the strain 84-183 chromosome by natural transformation, resulting in strain 94-49. Proper replacement of the wild-type *eno* allele with the Km^r mutagenized allele was verified by PCR (data not shown) and was not accompanied by insertion of vector sequences.

UV light and MMS sensitivities of *recA* and *eno H. pylori* strains. To test for loss of activity of the *H. pylori* RecA protein, we assayed the sensitivities of wild-type, *recA*, and *eno* strains to irradiation with 254-nm UV light or treatment with the DNA-damaging agent MMS (Fig. 4). *E. coli* Y1089 (*recA*$^+$) and DH5αMCR (*recA1*) were used as controls in this experiment as was a *recA*$^+$ *H. pylori* 84-183 derivative (93-225) in which the Km^r gene had been inserted into the *cagA* locus (65). *recA*$^+$ *H. pylori* 84-183 and 93-225 were similar in their susceptibilities to UV light, verifying that kanamycin resistance per se had no effect on survival. Both *recA*$^+$ *H. pylori* strains were somewhat more sensitive to killing by UV light than *recA*$^+$ *E. coli* (data not shown). Survival of *recA* mutant strains 93-226 and 93-227 was similar to that seen for *recA E. coli* (data not shown). The *eno* mutant 94-49 showed UV light sensitivity intermediate to those of wild-type and *recA H. pylori* strains. Parallel experiments were performed on *recA* mutants with exposure to MMS, with similar results (Fig. 4B). Both *recA H. pylori* strains showed greatly increased sensitivity to MMS relative to that of the two *recA*$^+$ *H. pylori* controls. Therefore, strains 93-226, 93-227, and, to some extent, 94-49 have phenotypes consistent with loss of RecA function.

Susceptibilities of *recA* and *eno* strains to antimicrobial agents. We examined the effect of the *recA* mutations in strains 93-226 and 93-227 on susceptibility to several antimicrobial agents (Table 3). As expected, the *recA* mutants were significantly more sensitive to metronidazole and ciprofloxacin, antimicrobial agents that cause DNA damage or interfere with replication (32, 59, 73). Interestingly, the *recA* strains also were significantly more sensitive to ampicillin, erythromycin, and tetracycline, which have different mechanisms of action. Strain 94-49 showed susceptibility to metronidazole (and to a lesser extent to ciprofloxacin) intermediate to those of the wild type and *recA* mutants. Susceptibilities to the remaining antimicrobial agents were similar to those observed with the *recA* mutants. These results suggest that two different phenomena may be responsible for the relative sensitivities of the *recA* and *eno* mutants to these antimicrobial agents. The increased sensitivity of the *eno* mutant to metronidazole (and possibly ciprofloxacin) might result from the decreased expression of *recA* in the *eno* mutant, while susceptibilities to the others may result from decreased expression of *eno* in both types of mutants.

TABLE 3. Activities of antimicrobial agents against wild-type, *recA*, and *eno H. pylori* strains

Antimicrobial agent	MIC (μg/ml)[a] of strain:			
	84-183[b]	93-226[c]	93-227[d]	94-49[e]
Ampicillin	0.016	<0.016	<0.016	<0.016
Ciprofloxacin	0.023	0.003	<0.002	0.005
Erythromycin	2	<0.016	<0.016	<0.016
Metronidazole	3	0.032	0.016	0.57
Tetracycline	0.125	<0.016	<0.016	<0.016

[a] As determined by E-test. Results given are the means of three (for strains 84-183, 93-226, and 93-227) or two (for strain 94-49) determinations.
[b] Wild-type (*recA*$^+$ *eno*$^+$) *H. pylori.*
[c] *recA*::*km H. pylori.*
[d] *recA*::*cm H. pylori.*
[e] *eno*::*km H. pylori.*

Acid sensitivity of *recA* and *eno* strains. Little is known about the exact environment in which *H. pylori* lives in the stomach. One condition that is certainly encountered is the low pH of gastric juice, and a role for the *H. pylori* urease protein in acid survival has been proposed (36, 38). To test whether *recA* or *eno* mutations had an effect on survival at low pH, we performed acid sensitivity experiments on the wild-type strain 84-183, the *recA* mutant 93-226, and the *eno* mutant 94-49 (Fig. 5). The experiments were performed in both the presence and absence of urea to differentiate between urease-dependent and urease-independent mechanisms. In the presence of urea at pH 4.0, survival of strain 93-226 was similar to that of strain 84-183. However, at pH 3.3 in the presence of urea, 93-226 was nearly 10-fold more sensitive to acid killing than 84-183. A similar difference in survival was seen at pH 4.0 in the absence of urea, indicating that the acid-sensitive phenotype in 93-226 was not due to interference with urease function. Similar to what was observed in sensitivities to UV light and metronidazole, the *eno* mutant 94-49 was killed by low pH to an extent intermediate between that of the wild-type strain and the *recA* mutant. The *recA* or *eno* strains did not survive incubation at pH 3.3 in the absence of urea, and therefore evaluation of survival relative to that of the wild type was not possible.

Detection of *recA* and *eno* mRNA by RT-PCR. To investigate directly whether insertion of an antibiotic resistance cassette into *recA* had polar effects on *eno* expression, we performed RT-PCR on RNA samples prepared from wild-type (84-183), *recA* (93-226), and *eno* (94-49) *H. pylori* strains. Crude RNA samples were first treated with RNase-free DNase to rid the preparations of contaminating DNA. Control PCR experi-

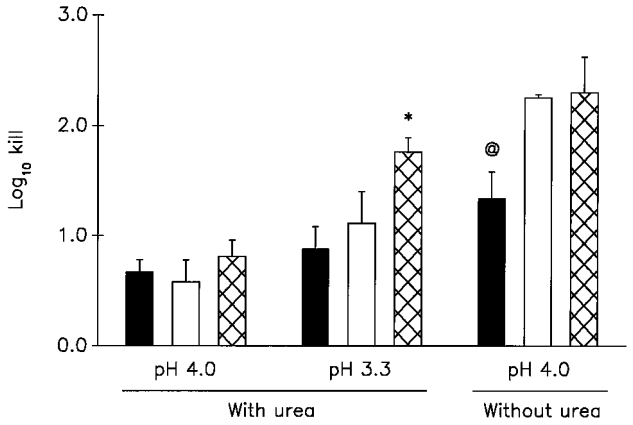

FIG. 5. Sensitivity of bacteria to low pH determined by comparing the $\log_{10}$ of the number of bacteria surviving incubation at low pH with the $\log_{10}$ of those surviving incubation at pH 7. Experiments were done in the presence or absence of urea (10 mM). Symbols: filled bars, *H. pylori* 84-183; open bars, *H. pylori* 94-49; cross-hatched bars, *H. pylori* 93-226. The results are the means of six experiments (for strains 84-183 and 93-226) or two experiments (for strain 94-49). The statistical significance of the killing of the mutants in comparison with that of the wild type is indicated by symbols above the appropriate bars. *, $P = 0.007$; @, $P = 0.03$.

ments verified that detectable DNA was removed by this treatment (data not shown). We then used cDNA prepared from these samples in PCR experiments with oligonucleotide primers specific for the *recA* and *eno* genes (Fig. 6). A primer pair encompassing the 3′ end of the *recA* gene and the 5′ end of the *eno* gene amplified the expected product in these *H. pylori* strains, supporting the DNA sequence data that the *recA* and *eno* genes are cotranscribed (Fig. 6B, lane 1). Significant transcription of *recA* occurred 3′ of the point of insertion of the Km^r cassette in *recA* mutant 93-226 (Fig. 6A, lane 2) and proceeded at least into the 5′ end of the *eno* gene (Fig. 6B, lane 2). However, the amount of transcript corresponding to the 3′ end of the cloned portion of the *eno* gene was greatly decreased in strain 93-226 (Fig. 6C, lane 2), suggesting that insertion of the Km^r cassette into *recA* exerted polar effects on production of a full-length *eno* transcript. In contrast to that seen with Km^r cassette insertion into *recA*, insertion of Km^r into *eno* did not result in appreciable transcription of downstream *eno* sequences (Fig. 6C, lane 3). Insertion of Km^r into *eno* also did not grossly affect the amount of *recA*-specific transcript (Fig. 6A, lane 3). Northern (RNA) hybridization supported the RT-PCR data and indicated that *recA* and *eno* are cotranscribed and that insertion of the Km^r cassette into

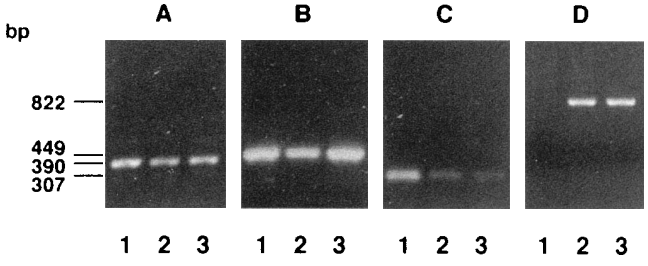

FIG. 6. RT-PCR of DNase-treated RNA extracted from strain 84-183 (lanes 1), 93-226 (lanes 2), or 94-49 (lanes 3). These cDNA samples were subjected to PCR with oligonucleotide primers specific for transcripts of *recA* (A), *recA-eno* (B), *eno* (C), and Km^r cassette (D). Primers used are listed in Table 2. The sizes of the PCR products are given in the left margin.

recA diminished the amount of *eno*-specific transcript (data not shown).

DISCUSSION

An understanding of the genetics of *H. pylori* is still at an early stage (64). In an attempt to begin elucidating genetic mechanisms of recombination, DNA transformation, and stress tolerance, we cloned the *H. pylori recA* gene. Much is known about the structure of RecA proteins of bacteria and related proteins from yeasts and bacteriophage T4 (54, 62). Alignment of RecA protein sequences allowed identification of invariant amino acid residues (62), and such strict amino acid conservation allowed the design of degenerate primers for use in PCR, as has been used previously for amplification of other *recA* homologs (12, 13). Not surprisingly, the length and mass of *H. pylori* RecA are similar to those of previously studied RecA proteins, and amino acid residues that are invariant in other RecA proteins also are conserved in *H. pylori* RecA. Typical −35 and −10 sequences constitute the probable promoter for *recA*. Unlike many SOS-regulated *recA* genes, a consensus LexA binding sequence ($CTGTN_9CAG$) (70) was not present in the predicted promoter region of *recA*. The termination codon for *recA* is separated by only 12 bp from the initiation codon of *eno*, and RT-PCR and Northern blot demonstrate that these genes are cotranscribed. Although a similar arrangement of *recA* and *eno* genes is found in *C. jejuni* (25), it is not immediately apparent why such seemingly unrelated genes should be on the same transcriptional unit, although the *eno* product may serve to couple the metabolism of the cell with DNA repair.

Insertional mutation of either gene on the *recA-eno* transcript appears to have the effect of decreasing the expression of the other gene. Insertion of the Km^r cassette into the *recA* gene in strain 93-226 clearly has polar effects on *eno* expression, although significant transcription (possibly from the Km^r promoter [8, 35]) occurs into the 3′ region of *recA* and the 5′ end of *eno* (Fig. 6A and B, lanes 2). It appears that a large portion of this transcription terminates between the end of the Eno104 PCR primer (nucleotide 1500) and the end of the Eno-R PCR primer (nucleotide 1933), since the amount of transcript detected in the more-distal region of *eno* appears to be significantly reduced (Fig. 6C, lane 2) in this mutant. It is therefore possible that the polarity of the Km^r cassette insertion on *eno* expression is due to termination of transcription at an RNA stem-loop structure near the beginning of *eno* corresponding to nucleotides 1570 to 1598 of the DNA sequence (Fig. 2). It may be that this stem-loop is active in terminating transcription in the wild-type strain as well and could provide a means of allowing increased expression of *recA* relative to *eno*. A similar arrangement of *recA* and *eno* was recently reported for *C. jejuni* (25). Although not addressed experimentally by those authors, we suspect that cotranscription of *recA* and *eno* also occurs in *C. jejuni* and that insertional mutation of *recA* may exert polar effects on *eno* expression. No problems were encountered in recovering *H. pylori recA* or *eno* mutants, and these strains do not appear to have serious growth defects. In *E. coli*, *eno* mutants do not grow well in the presence of glycolytic sugars (31). This may represent a basic difference between *E. coli* and *H. pylori* carbohydrate metabolism, although the glycolytic pathway has recently been described for *H. pylori* (44). The *eno* mutant described here may be interesting for further study on the utilization of carbohydrates by *H. pylori*.

Interestingly, it appears that insertional disruption of *eno* also affects expression of the upstream *recA* gene, possibly via a mechanism involving decreased stability of the altered *recA*-

eno transcript (47) (see below). The effects on neighboring gene expression of mutating the *recA* or *eno* genes as well as the unavailability of reliable *H. pylori* shuttle vectors for complementation studies make it difficult to assign with any certainty a given mutant phenotype to either *recA* or *eno*. However, some insight may be obtained by examining phenotypes highly characteristic of *recA* mutation in other bacteria. Phenotypes related to DNA damage are more likely to be due to alteration of *recA* expression. This suggests, although certainly not conclusively, that the increased sensitivities of the *eno* mutant to UV light and metronidazole are due to decreased expression of *recA* rather than to loss of the *eno* product. The same argument might be applied to sensitivity to killing by low pH, since in these experiments the *eno* mutants also show susceptibilities intermediate to those of wild-type and *recA* strains. However, since little is known in any bacterial system about the cellular damage caused by low pH or the specific mechanisms of acid resistance, this extrapolation remains to be proven. *H. pylori* urease converts physiologic levels of urea to ammonia and has been shown to increase survival of *H. pylori* at acidic pH (42, 43, 49), probably by buffering the microenvironment of the organism. As the extracellular pH drops, however, the buffering capacity of ammonia produced by urease may be exceeded, resulting in lowering of the pH of the bacterial cytoplasm. Significant lowering of the intracellular pH may result in DNA damage via depurination, and in *Salmonella typhimurium*, a DNA repair protein (PolA) is induced by exposure to low pH (16). RecA may have a direct role in recombinational repair of such lesions or an indirect role by stimulating an SOS-like response.

The RecA protein is a ubiquitous bacterial recombination protein which is required for DNA transformation of some bacteria (51, 69) and which also has a central role in mediating bacterial SOS responses (70). In *E. coli*, the SOS regulon consists of greater than 20 genes and is induced when the cell encounters agents which damage DNA or inhibit cell division (70). RecA becomes activated following DNA damage and signals the cleavage of the LexA repressor, which normally binds a conserved sequence in the promoters of SOS genes, including *recA* itself. Cleaved LexA is unable to bind SOS promoters, resulting in induction of SOS genes (70). This response to DNA damage is probably widespread in bacteria, and SOS genes other than those in *E. coli* have been identified; these genes contain LexA-binding sequences in their promoter regions. Not all cloned *recA* genes have identifiable LexA binding sites (9, 11, 19, 23, 24, 45, 52, 53, 63, 72), and it is not known whether this represents a different form of regulation of *recA* in those bacteria. The lack of a consensus LexA binding site in the *H. pylori recA* promoter may have implications regarding the regulation of a putative SOS-like response in *H. pylori*. It is possible that *H. pylori* has a LexA homolog but one that binds a different sequence in the promoters of *H. pylori* SOS genes. Alternatively, a different protein could serve the same function as LexA. Another possibility is that LexA regulation functions as it does in *E. coli* but that in *H. pylori recA* is not itself a part of the SOS regulon. Answers to these questions await further experimentation.

It is probable that *H. pylori* organisms are exposed to many types of stress when infecting the human stomach. Among these are the nonspecific antibacterial factors (e.g., low pH) of gastric juice and reactive oxidative molecules released by immune cells during inflammation of the gastric mucosa (68). Some of these types of stress cause DNA damage (26, 29) and therefore may induce a response in *H. pylori* similar to that of SOS (21). Management of environmental stress is certainly important for survival of *H. pylori* in the stomach; any responses that contribute to this are of interest. Superoxide dismutase (SodB) and catalase activities have been reported for *H. pylori* (30, 60). These also may play a role in resistance to oxidative DNA damage (28, 29) and could be linked to an SOS response.

Since infection with *H. pylori* is so prevalent worldwide and is associated with significant morbidity, there is great interest in a vaccine against *H. pylori*. It is possible that mutation of *recA* would be of use in a vaccine strain, as has been suggested previously for *Vibrio cholerae* (22), for several reasons. First, lack of RecA function may afford increased genetic stability to other markers that are necessary for a vigorous immune response. For example, the CagA immunodominant antigen undergoes size variation in vitro (66). If expression of a certain CagA size variant is important in achieving a protective immune response, mutation of *recA* may prevent variation to a different size. Second, *recA* mutation is likely to greatly diminish the DNA transformation frequency of *H. pylori* (25, 33, 51, 69). Most strains of *H. pylori* are naturally competent for DNA uptake (71), and it is possible that DNA transformation occurs in vivo. Indeed, our preliminary data suggest that our *H. pylori recA* mutant (strain 93-227) was completely deficient in the ability to be transformed by a DNA fragment that contained a Km^r cassette insertion in the *cagA* gene (data not shown). Inhibition of transformation via *recA* mutation would be desirable, preventing reversion of an attenuated strain to virulence through uptake of DNA from resident *H. pylori* organisms. Similarly, *recA* mutation should prevent reversions based on homologous recombination involving endogenous sequences. Third, the enhanced susceptibilities of these *recA* strains to antimicrobial agents make them attractive as candidates for live vaccine strains, since this would facilitate their eradication upon completion of a vaccination regimen; the heightened (approximately 2-log) susceptibility to metronidazole may be particularly useful. Fourth, the enhanced susceptibility to low pH suggests that the tenure of a *recA* mutant in a normal host would not be lifelong but, possibly, substantially briefer. In total, these qualities suggest that *recA* mutation is an essential characteristic of live attenuated *H. pylori* vaccine strains. Finally, if an attenuated *H. pylori* strain is ever used as a carrier of heterologous proteins, a *recA* mutant would also be useful.

ACKNOWLEDGMENTS

We thank Murali Tummuru for many helpful discussions and Joel Dworkin for the donation of degenerate *recA* and Km^r cassette PCR primers.

This work was supported by National Institutes of Health grant R01CA58834 and by the Medical Research Service of the Department of Veterans Affairs.

REFERENCES

1. **Akopyanz, N., N. O. Bukanov, T. U. Westblom, and D. E. Berg.** 1992. PCR-based RFLP analysis of DNA sequence diversity in the gastric pathogen *Helicobacter pylori*. Nucleic Acids Res. **20:**6221–6225.
2. **Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.** 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
3. **Biswas, G. D., J. Graves, R. Schwalbe, and P. F. Sparling.** 1986. Construction of isogenic gonococcal strains varying in the presence of a 4.2-kilobase cryptic plasmid. J. Bacteriol. **167:**685–694.
4. **Blaser, M. J., and J. Parsonnet.** 1994. Parasitism by the "slow" bacterium *Helicobacter pylori* leads to altered gastric homeostasis and neoplasia. J. Clin. Invest. **94:**4–8.
5. **Buchmeier, N. A., C. J. Lipps, M. Y. H. So, and F. Heffron.** 1993. Recombination-deficient mutants of *Salmonella typhimurium* are avirulent and sensitive to the oxidative burst of macrophages. Mol. Microbiol. **7:**933–936.
6. **Clayton, C. L., H. Kleanthous, D. D. Morgan, L. Puckey, and S. Tabaqchali.** 1993. Rapid fingerprinting of *Helicobacter pylori* by polymerase chain reac-

tion and restriction fragment length polymorphism analysis. J. Clin. Microbiol. **31:**1420–1425.
7. **Cover, T. L., C. P. Dooley, and M. J. Blaser.** 1990. Characterization of and human serologic response to proteins in *Helicobacter pylori* broth culture supernatants with vacuolizing cytotoxin activity. Infect. Immun. **58:**603–610.
8. **Cussac, V., R. L. Ferrero, and A. Labigne.** 1992. Expression of *Helicobacter pylori* urease genes in *Escherichia coli* grown under nitrogen-limiting conditions. J. Bacteriol. **174:**2466–2473.
9. **Davis, E. O., S. G. Sedgwick, and M. J. Colston.** 1991. Novel structure of the *recA* locus of *Mycobacterium tuberculosis* implies processing of the gene product. J. Bacteriol. **173:**5653–5662.
10. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
11. **Dunkin, S. M., and D. O. Wood.** 1994. Isolation and characterization of the *Rickettsia prowazekii recA* gene. J. Bacteriol. **176:**1777–1781.
12. **Duwat, P., S. D. Ehrlich, and A. Gruss.** 1992. A general method for cloning *recA* genes of gram-positive bacteria by polymerase chain reaction. J. Bacteriol. **174:**5171–5175.

12a. **Dworkin, J.** Unpublished results.

13. **Dybvig, K., S. K. Hollingshead, D. G. Heath, D. B. Clewell, F. Sun, and A. Woodard.** 1992. Degenerate oligonucleotide primers for enzymatic amplification of *recA* sequences from gram-positive bacteria and mycoplasmas. J. Bacteriol. **174:**2729–2732.
14. **Eaton, K. A., D. R. Morgan, and S. Krakowka.** 1992. Motility as a factor in the colonisation of gnotobiotic piglets by *Helicobacter pylori*. J. Med. Microbiol. **37:**123–127.
15. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132:**6.
16. **Foster, J. W., and B. Bearson.** 1994. Acid-sensitive mutants of *Salmonella typhimurium* identified through a dinitrophenol lethal screening strategy. J. Bacteriol. **176:**2596–2607.
17. **Foxall, P. A., L. T. Hu, and H. L. Mobley.** 1992. Use of polymerase chain reaction-amplified *Helicobacter pylori* urease structural genes for differentiation of isolates. J. Clin. Microbiol. **30:**739–741.
18. **Fujimoto, S., B. Marshall, and M. J. Blaser.** 1994. PCR-based restriction fragment polymorphism typing of *Helicobacter pylori*. J. Clin. Microbiol. **32:**331–334.
19. **Fyfe, J. A., and J. K. Davies.** 1990. Nucleotide sequence and expression in *Escherichia coli* of the *recA* gene of *Neisseria gonorrhoeae*. Gene **93:**151–156.
20. **Gish, W., and D. J. States.** 1993. Identification of protein coding regions by database similarity search. Nat. Genet. **3:**266–272.
21. **Goehrlich, O., P. Quillardet, and M. Hofnung.** 1989. Induction of the SOS response by hydrogen peroxide in various *Escherichia coli* mutants with altered protection against oxidative damage. J. Bacteriol. **171:**6141–6147.
22. **Goldberg, I., and J. Mekalanos.** 1986. Cloning of the *Vibrio cholerae recA* gene and construction of a *Vibrio cholerae recA* mutant. J. Bacteriol. **165:** 715–722.
23. **Gomelsky, M., E. Gak, A. Chistoserdov, A. Bolotin, and Y. D. Tsygankov.** 1990. Cloning, sequence and expression in *Escherichia coli* of the *Methylobacillus flagellatum recA* gene. Gene **94:**69–75.
24. **Goodman, H. J., and D. R. Woods.** 1990. Molecular analysis of the *Bacteroides fragilis recA* gene. Gene **94:**77–82.
25. **Guerry, P., P. M. Pope, D. H. Burr, J. Leifer, S. W. Joseph, and A. L. Bourgeois.** 1994. Development and characterization of *recA* mutants of *Campylobacter jejuni* for inclusion in attenuated vaccines. Infect. Immun. **62:**426–432.
26. **Haas, A., and W. Goebel.** 1992. Microbial strategies to prevent oxygen-dependent killing by phagocytes. Free Radical Res. Commun. **16:**137–157.
27. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557.
28. **Hassett, D. J., L. Charniga, K. Bean, D. E. Ohman, and M. S. Cohen.** 1992. Response of *Pseudomonas aeruginosa* to pyocyanin: mechanisms of resistance, antioxidant defenses, and demonstration of a manganese-cofactored superoxide dismutase. Infect. Immun. **60:**328–336.
29. **Hassett, D. J., and M. S. Cohen.** 1989. Bacterial adaptation to oxidative stress: implications for pathogenesis and interaction with phagocytic cells. FASEB J. **3:**2574–2582.
30. **Hazell, S. L., D. J. Evans, and D. Y. Graham.** 1991. *Helicobacter pylori* catalase. J. Gen. Microbiol. **137:**57–61.
31. **Irani, M. H., and P. K. Maitra.** 1977. Properties of *Escherichia coli* mutants deficient in enzymes of glycolysis. J. Bacteriol. **132:**398–410.
32. **Knight, R. C., I. M. Skolimowski, and D. I. Edwards.** 1978. The interaction of reduced metronidazole with DNA. Biochem. Pharmacol. **27:**2089–2093.
33. **Koomey, J. M., and S. Falkow.** 1987. Cloning of the *recA* gene of *Neisseria gonorrhoeae* and construction of gonococcal *recA* mutants. J. Bacteriol. **169:** 790–795.
34. **Kraft, R., J. Tardiff, K. S. Krauter, and L. A. Leinwand.** 1988. Using miniprep plasmid DNA for sequencing double stranded templates with Sequenase. Biotechniques **6:**544–546.
35. **Labigne, A.** Personal communication.
36. **Labigne, A., V. Cussac, and P. Courcoux.** 1991. Shuttle cloning and nucleotide sequences of *Helicobacter pylori* genes responsible for urease activity. J. Bacteriol. **173:**1920–1931.
37. **Labigne-Roussel, A., P. Courcoux, and L. Tompkins.** 1988. Gene disruption and replacement as a feasible approach for mutagenesis of *Campylobacter jejuni*. J. Bacteriol. **170:**1704–1708.
38. **Lee, A., J. Fox, and S. Hazell.** 1993. Pathogenicity of *Helicobacter pylori*: a perspective. Infect. Immun. **61:**1601–1610.
39. **Leunk, R. D., P. T. Johnson, B. C. David, W. G. Kraft, and D. R. Morgan.** 1988. Cytotoxic activity in broth-culture filtrates of *Campylobacter pylori*. J. Med. Microbiol. **26:**93–99.
40. **Leying, H., S. Suerbaum, G. Geis, and R. Haas.** 1992. Cloning and genetic characterization of a *Helicobacter pylori* flagellin gene. Mol. Microbiol. **6:**2863–2874.
41. **Lopez, C. R., R. J. Owen, and M. Desai.** 1993. Differentiation between isolates of *Helicobacter pylori* by PCR-RFLP analysis of urease A and B genes and comparison with ribosomal RNA gene patterns. FEMS Microbiol. Lett. **110:**37–43.
42. **Marshall, B. J., L. J. Barrett, K. Prakash, R. W. McCallum, and R. L. Guerrant.** 1990. Urea protects *Helicobacter* (*Campylobacter*) *pylori* from the bactericidal effect of acid. Gastroenterology **99:**697–702.
43. **McGowan, C. C., T. L. Cover, and M. J. Blaser.** 1994. The proton pump inhibitor, omeprazole, inhibits acid survival of *Helicobacter pylori* by a urease-independent mechanism. Gastroenterology **107:**1573–1578.
44. **Mendz, G. L., S. L. Hazell, and B. P. Burns.** 1993. Glucose utilization and lactate production by *Helicobacter pylori*. J. Gen. Microbiol. **139:**3023–3028.
45. **Michiels, J., A. Vande Broek, and J. Vanderleyden.** 1991. Molecular cloning and nucleotide sequence of the *Rhizobium phaseoli recA* gene. Mol. Gen. Genet. **228:**486–490.
46. **Mobley, H. L., L. T. Hu, and P. A. Foxal.** 1991. *Helicobacter pylori* urease: properties and role in pathogenesis. Scand. J. Gastroenterol. Suppl. **187:**39–46.
47. **Newbury, S. F., N. H. Smith, and C. F. Higgins.** 1987. Differential mRNA stability controls relative gene expression within a polycistronic operon. Cell **51:**1131–1143.
48. **Peek, R. M., G. G. Miller, and M. J. Blaser.** Reverse transcription-polymerase chain reaction (RT-PCR) for bacterial and host gene expression in human gastric mucosa. *In* F. Megraud, N. Lee, and A. Lee (ed.), *Helicobacter pylori* techniques for clinical diagnosis and basic research, in press. W.B. Saunders, Ltd., Philadelphia.
49. **Pérez-Pérez, G., A. Z. Olivares, T. L. Cover, and M. J. Blaser.** 1992. Characteristics of *Helicobacter pylori* variants selected for urease deficiency. Infect. Immun. **60:**3658–3663.
50. **Pérez-Pérez, G. I., and M. J. Blaser.** 1987. Conservation and diversity of *Campylobacter pyloridis* major antigens. Infect. Immun. **55:**505–513.
51. **Quivey, R. J., and R. C. Faustoferri.** 1992. In vivo inactivation of the *Streptococcus mutans recA* gene mediated by PCR amplification and cloning of a *recA* DNA fragment. Gene **116:**35–42.
52. **Ramesar, R. S., V. Abratt, D. R. Woods, and D. E. Rawlings.** 1989. Nucleotide sequence and expression of a cloned *Thiobacillus ferrooxidans recA* gene in *Escherichia coli*. Gene **78:**1–8.
53. **Ridder, R., R. Marquardt, and K. Esser.** 1991. Molecular cloning and characterization of the *recA* gene of *Methylomonas clara* and construction of a *recA* deficient mutant. Appl. Microbiol. Biotechnol. **35:**23–31.
54. **Roca, A. I., and M. M. Cox.** 1990. The RecA protein: structure and function. Crit. Rev. Biochem. Mol. Biol. **25:**415–456.
55. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1987. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
56. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
57. **Schleif, R. F., and P. C. Wensink.** 1981. Practical methods in molecular biology, p. 98–105. Springer-Verlag, New York.
58. **Segal, E. D., and L. S. Tompkins.** 1993. Transformation of *Helicobacter pylori* by electroporation. Biotechniques **14:**225–226.
59. **Sioud, M., and P. Forterre.** 1989. Ciprofloxacin and etoposide (VP16) produce a similar pattern of DNA cleavage in a plasmid of an archaebacterium. Biochemistry **28:**3638–3641.
60. **Spiegelhalder, C., B. Gerstenecker, A. Kersten, E. Schiltz, and M. Kist.** 1993. Purification of *Helicobacter pylori* superoxide dismutase and cloning and sequencing of the gene. Infect. Immun. **61:**5315–5325.
61. **Staden, R.** 1982. Automation of the computer handling of gel reading data produced by the shotgun method of DNA sequencing. Nucleic Acids Res. **10:**4731–4751.
62. **Story, R. M., D. K. Bishop, N. Kleckner, and T. A. Steitz.** 1993. Structural relationship of bacterial RecA proteins to recombination proteins from bacteriophage T4 and yeast. Science **259:**1892–1896.
63. **Tatum, F. M., D. C. Morfitt, and S. M. Halling.** 1993. Construction of a *Brucella abortus* RecA mutant and its survival in mice. Microb. Pathog. **14:**177–185.
64. **Taylor, D. E.** 1992. Genetics of *Campylobacter* and *Helicobacter*. Annu. Rev. Microbiol. **46:**35–64.
65. **Thompson, S. A.** Unpublished data.
66. **Tummuru, M. K.** Personal communication.

67. **Tummuru, M. K., T. L. Cover, and M. J. Blaser.** 1993. Cloning and expression of a high-molecular-mass major antigen of *Helicobacter pylori*: evidence of linkage to cytotoxin production. Infect. Immun. **61:**1799–1809.
68. **Tytgat, G. N.** 1992. Does the stomach adapt to *Helicobacter pylori*? Scand. J. Gastroenterol. Suppl. **193:**28–32.
69. **Vosman, B., and K. J. Hellingwerf.** 1991. Molecular cloning and functional characterization of a *recA* analog from *Pseudomonas stutzeri* and construction of a *P. stutzeri recA* mutant. Antonie Leeuwenhoek **59:**115–123.
70. **Walker, G. C.** 1984. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol. Rev. **48:**60–93.
71. **Wang, Y., K. P. Roos, and D. E. Taylor.** 1993. Transformation of *Helicobacter pylori* by chromosomal metronidazole resistance and by a plasmid with a selectable chloramphenicol resistance marker. J. Gen. Microbiol. **139:**2485–2493.
72. **Wardhan, H., M. J. McPherson, C. A. Harris, E. Sharma, and G. R. Sastry.** 1992. Molecular analysis of the *recA* gene of *Agrobacterium tumefaciens* C58. Gene **121:**133–136.
73. **Wolfson, J. S., and D. C. Hooper.** 1985. The fluoroquinolones: structures, mechanisms of action and resistance, and spectra of activity in vitro. Antimicrob. Agents Chemother. **28:**581–586.
74. **Yao, R., R. A. Alm, T. J. Trust, and P. Guerry.** 1993. Construction of new *Campylobacter* cloning vectors and a new mutational *cat* cassette. Gene **130:**127–130.
75. **Young, R. A., and R. W. Davis.** 1983. Efficient isolation of genes by using antibody probes. Proc. Natl. Acad. Sci. USA **80:**1194–1198.