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The RecA Protein of *Helicobacter pylori* Requires a Posttranslational Modification for Full Activity

Wolfgang Fischer* and Rainer Haas

Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, Ludwig-Maximilians-Universität, D-80336 Munich, Germany

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The RecA protein is a central component of the homologous recombination machinery and of the SOS system in most bacteria. In performing these functions, it is involved in DNA repair processes and plays an important role in natural transformation competence. This may be especially important in *Helicobacter pylori*, where an unusually high degree of microdiversity among strains is generated by homologous recombination. We have suggested previously that the *H. pylori* RecA protein is subject to posttranslational modifications that result in a slight shift in its electrophoretic mobility. Here we show that at least two genes downstream of *recA* are involved in this modification and that this process is dependent on genes involved in glycosylation and lipopolysaccharide biosynthesis. Site-directed mutagenesis of a putative glycosylation site results in production of an unmodified RecA protein. This posttranslational modification is not involved in membrane targeting or cell division functions but is necessary for the full function of RecA in DNA repair. Thus, it might be an adaptation to the specific requirements of *H. pylori* in its natural environment.

The gram-negative bacterial pathogen *Helicobacter pylori* is the principal cause of chronic active gastritis and peptic ulcer disease and has been implicated in the development of gastric mucosa-associated lymphoid tissue lymphoma and gastric cancer (29, 40). The very special habitat of *H. pylori* at the surface of gastric epithelial cells or in the mucus layer covering the epithelium suggests that this bacterium has evolved specialized features for adaptation. A comparison of the two published genome sequences shows a considerable diversity in gene content, with about 7% of all putative genes being strain specific (1). Moreover, some *H. pylori* strains display high mutation frequencies (3), and more diversity is created by horizontal gene transfer and free recombination between strains (41), although infections with multiple strains are not very common. Since *H. pylori* is naturally competent for transformation, horizontal gene transfer is supposed to occur mainly by this mechanism.

Thus, homologous recombination is an important function which helps to generate diversity, but it is also involved in maintaining genome integrity and thus species barriers (28). These functions are achieved by a complex machinery which is highly regulated and which involves many proteins (9). The RecA protein is one of the central components of this machinery. One of its main functions is the recognition of stretches of single-stranded DNA, which are subsequently complexed by helical RecA filaments. In *Escherichia coli*, this filamentous form of RecA is activated as a coprotease that regulates cellular functions such as the SOS response or the induction of *trans*-lesion DNA synthesis. Both this coprotease activity and the recombination function are thought to play major roles in the bacterial response to DNA damage. The RecA protein of *H. pylori* has likewise been shown to be necessary for DNA repair (36, 44), although SOS response and error-prone *trans*-lesion synthesis pathways do not seem to be present in *H. pylori*, as concluded by the absence of homologous genes (10).

Here we describe a posttranslational protein modification of the RecA protein in *H. pylori*, and we show that this modification is necessary for the full function of RecA in response to DNA damage.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *H. pylori* strains were grown on GC agar plates (Difco) supplemented with vitamin mix (1%), horse serum (8%), vancomycin (10 mg/liter), trimethoprim (5 mg/liter), and nystatin (1 mg/liter) (serum plates) and incubated for 16 to 60 h in a microaerobic atmosphere (85% N_2, 10% CO_2, 5% O_2) at 37°C. *E. coli* strains HB101 (4), GC6 (43), and DH5α (Bethesda Research Laboratories) were grown on Luria-Bertani agar plates or in Luria-Bertani liquid medium (33) supplemented with ampicillin (100 mg/liter), chloramphenicol (30 mg/liter), or kanamycin (40 mg/liter), as appropriate. Strains β2150 and β2155 (6) were grown on the same media supplemented with diaminopimelic acid (0.2 mM).

DNA manipulations. Standard cloning and DNA analysis procedures were performed according to the methods described in reference 33. Plasmid DNA was purified from *E. coli* by the boiling procedure, and *E. coli* cells for electroporation were prepared according to the protocol recommended for the Gene Pulser (Bio-Rad). Plasmid DNA was isolated from *Helicobacter* strains by using Wizard minipreps (Promega) according to the protocol of the manufacturer. Amplification of DNA fragments by PCR was performed as described previously (15).

Natural transformation and bacterial conjugation. Shuttle plasmids and suicide plasmids were introduced into *H. pylori* strains by conjugation or transformation as described previously (16), except that in conjugation experiments *E. coli* strains β2150 (for plasmids conferring kanamycin resistance) and β2155 (for plasmids conferring chloramphenicol resistance) were used as donors and β2150(pRK2013) was used as a mobilizing strain. *H. pylori* transformants were selected on serum plates containing 6 mg of chloramphenicol/liter or 8 mg of kanamycin/liter. For the determination of transformation rates, DNase (1 mg/ml) was added to *H. pylori* grown in liquid culture 1 h after the addition of plasmid DNA. The cultures were incubated for a further 6 to 12 h and then plated on selective agar plates and in parallel on nonselective agar plates to estimate the number of viable bacteria.

* Corresponding author. Mailing address: Max von Pettenkofer-Institut für Hygiene und Medizinische Mikrobiologie, Ludwig-Maximilians-Universität, Pettenkoferstr. 9a, D-80336 Munich, Germany. Phone: 49-89-51605277. Fax: 49-89-51605223. E-mail: fischer@m3401.mpk.med.uni-muenchen.de.

Plasmid constructions. Plasmid pDH38, which was used for the complementation of an *H. pylori recA* deletion mutant, was described earlier (36). For the construction of the enolase mutants, an *Eco*RI/*Bam*HI deletion derivative of plasmid pWS48, which contains the *recA-eno* region of *H. pylori* strain P1 (36), was constructed. The enolase gene was disrupted by replacing an internal 260-bp *Sph*I fragment with an *aphA-3* resistance gene cassette, resulting in plasmid pWS55, or with a terminatorless *cat* resistance gene cassette, resulting in plasmid pGAH3. Plasmid pWS106 was constructed by cloning the *recA* gene amplified with primers WS67 (5′-GAAGATCTTATTCCATTTCTTCTAAAG-3′) and WS68 (5′-CGGAATTCGCAATAGATGAAGACAAAC-3′) into the *Eco*RI and *Bam*HI sites of the expression vector pEV40 (31). The *recA-eno* downstream region comprising genes *hp155* to *hp158* was amplified with primers ET10 (5′-CGCGGATCCAAGAGTTGTTTAAGCATGGC-3′) and ET11 (5′-TAATGC ACTGCAGCCCACAATACGACAAAATC-3′) from chromosomal DNA of strain 26695 and cloned into the *Pst*I and *Bam*HI sites of pBluescript II KS (Stratagene). From there, the *recA-eno* downstream region was subcloned into the *Bgl*II and *Xho*I sites of plasmid pMin1 (22), resulting in plasmid pWS124. The site-specific mutation was introduced into the *recA* gene as follows. A 5′ portion of the *recA* gene of strain P1 was amplified by PCR with primers RH147 (5′-AGCTGGGTCGACTTTCTTAACGCGTGGCTC-3′) and DH13 (5′-GGG GTACCAAGCTTATCGCGCTCACATC-3′) and cloned into the *Kpn*I and *Sal*I sites of vector pIC20R1 (26), resulting in plasmid pGAH4. A 3′ portion of the *recA* gene containing the site-specific mutation was amplified with primers WS86 (5′-GCTCTAGACTGCAGAGATCAAAGGATCTTCTT-3′) and WS87 (5′-A ACGGACGCGTTAAGAAAAATCACCGGTGTTTTGCACAAAATGAATA CTATG-3′) and cloned into the *Mlu*I and *Bgl*II sites of plasmid pGAH4. The mutagenized *recA* gene was excised from the resulting plasmid pWS126 and subcloned into the *Kpn*I and *Bgl*II sites of the shuttle plasmid pHel3 (16) to yield plasmid pWS127.

Transposon shuttle mutagenesis. Tn*Max5* transposon mutagenesis of plasmid pWS124 was performed as described previously (22).

Production of anti-RecA antiserum and immunoblotting. A His_6-tagged RecA fusion protein derived from strain P1 was overproduced in *E. coli* 2136 from plasmid pWS106 and purified from inclusion bodies according to the method described in reference 39. The purified fusion protein was used to raise the polyclonal rabbit antiserum AK263. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting were performed as described previously (36). For the development of Western blots, nitrocellulose filters were blocked with 3% bovine serum albumin in TBS (50 mM Tris-HCl [pH 7.5], 150 mM NaCl) and incubated with AK263 at a dilution of 1:3,000. Protein A-conjugated alkaline phosphatase was used to visualize bound antibody.

Membrane preparations. *H. pylori* cells were grown on solid or in liquid media for 24 to 48 h, then harvested, washed, and resuspended in preparation buffer (10 mM Tris-HCl, pH 8.0). Bacteria were lysed by sonication, centrifuged for 10 min at 7,000 × *g* to remove unbroken cells and cell debris. The supernatant was collected and separated by ultracentrifugation (45 min, 230,000 × *g*) into cytoplasmic and total membrane fractions. Proteins in the cytoplasmic fractions were concentrated by chloroform-methanol precipitation (46), and the membrane fractions were washed with preparation buffer and resuspended in SDS-PAGE sample solution.

UV and metronidazole sensitivity measurements. The susceptibility of *H. pylori* strains to UV radiation was determined as described previously (36). For the determination of metronidazole sensitivity, E-test strips (AB Biodisk, Solna, Sweden) were placed on serum agar plates inoculated with standard *H. pylori* suspensions according to the method described in reference 44. MICs were scored after 5 days of growth.

Nucleoid staining and microscopy. Bacteria grown in liquid culture were harvested by centrifugation, washed twice, and resuspended in phosphate-buffered saline (PBS). The suspension was centrifuged onto coverslips (5 min, 1,500 × *g*) and fixed with 3.7% paraformaldehyde in PBS for 30 min at room temperature. After two washes with PBS, coverslips were incubated for 30 min in PBS containing 1 μg of 4′,6′-diamidino-2-phenylindole (DAPI)/ml, washed twice with PBS, and placed upon a slide containing a single drop of Fluoprep (BioMérieux). Cells were photographed with a Leica DM fluorescence microscope equipped with a Diagnostic Instruments SP401-220 digital camera.

RESULTS

Size variation of the *H. pylori* RecA protein. The *recA* gene in *H. pylori* encodes a protein with a calculated size of 37.6 kDa. During previously published complementation studies (36), it was noticed that *H. pylori* RecA migrates at an apparent size of 38 kDa when expressed from the shuttle plasmid pDH38 in *E. coli* but at a size of 40 kDa when the same shuttle plasmid was used in *H. pylori* (Fig. 1). Thus, we suspected that *H. pylori* RecA might be subject to a posttranslational modification. RecA is a highly conserved protein among bacterial species, and in many cases, bacterial *recA* genes are capable of complementing an *E. coli recA* phenotype (for examples, see references 12 and 23). Since the *H. pylori recA* gene was not able to do so (despite a protein sequence similarity of 74% and identity of 62%), we also suspected that this posttranslational modification might be necessary for the function of the *H. pylori* RecA protein (36).

Production of an unmodified RecA protein in *H. pylori*. The *recA* gene in *H. pylori* is followed downstream by a gene encoding an enolase homologue (*eno*). The two genes are cotranscribed, and mutations in the *eno* gene have been shown to result in a slightly increased sensitivity of the bacteria to DNA-damaging agents, which was attributed to an effect on *recA* expression (44). Therefore, we constructed an enolase mutant by inserting a kanamycin resistance cassette into the *eno* gene of strain P1 (Fig. 1A) to detect effects on RecA production and/or size. The P1 *eno* strain did not produce lower amounts of RecA protein, as the wild-type P1 strain did, as estimated by immunoblotting (Fig. 1B). But the RecA protein seems to be unmodified in the P1 *eno* strain; its electrophoretic mobility is higher than in the wild-type and seems to be the same as in the *E. coli* strain GC6(pDH38) expressing *H. pylori recA*. Since mutations in the enolase gene have been reported to confer enhanced sensitivity to DNA-damaging agents (44), we were interested in the phenotypes of the enolase mutant. We were, however, unable to detect a difference between the wild type and the enolase mutant regarding transformation competence or sensitivity to UV radiation (data not shown). The only obvious phenotype of the enolase mutant was its growth in an elongated cell shape, which is presumably due to a cell division defect (see below).

Involvement of other genes in the *recA* locus. In the two published *H. pylori* genome sequences, the *recA* gene is the first gene of a putative operon containing, in addition to *eno*, a shikimate kinase gene homologue (*aroK*) and three open reading frames with unknown function (Fig. 2A). Two of these open reading frames (*hp155* and *hp156*) have homologues in *Campylobacter jejuni* (*cj1671c* and *cj1670c*, respectively), which are also located downstream of the *recA* gene there (Fig. 2A). The third open reading frame (*hp158*) displays significant homology to a *C. jejuni* gene termed *pglG*, which is part of a gene locus involved in protein glycosylation in *C. jejuni* (42). We reasoned that in *H. pylori* the putative operon structure of the *recA* locus might reflect a functional correlation between the corresponding gene products. Therefore, we decided to examine the role of mutations in *recA-eno* downstream genes on the production or function of RecA. The *recA-eno* downstream region was amplified by PCR and cloned into the minimal vector pMin1, resulting in plasmid pWS124. Using this plasmid, the *recA-eno* downstream region was subjected to Tn*Max5* transposon mutagenesis. Transposon insertions were mapped to the reading frames *hp156* and *hp158* (Fig. 2A), and corresponding *H. pylori* P1 mutants were generated by natural transformation. Immunoblotting experiments with antiserum AK263 showed that in all of these insertion mutants the RecA

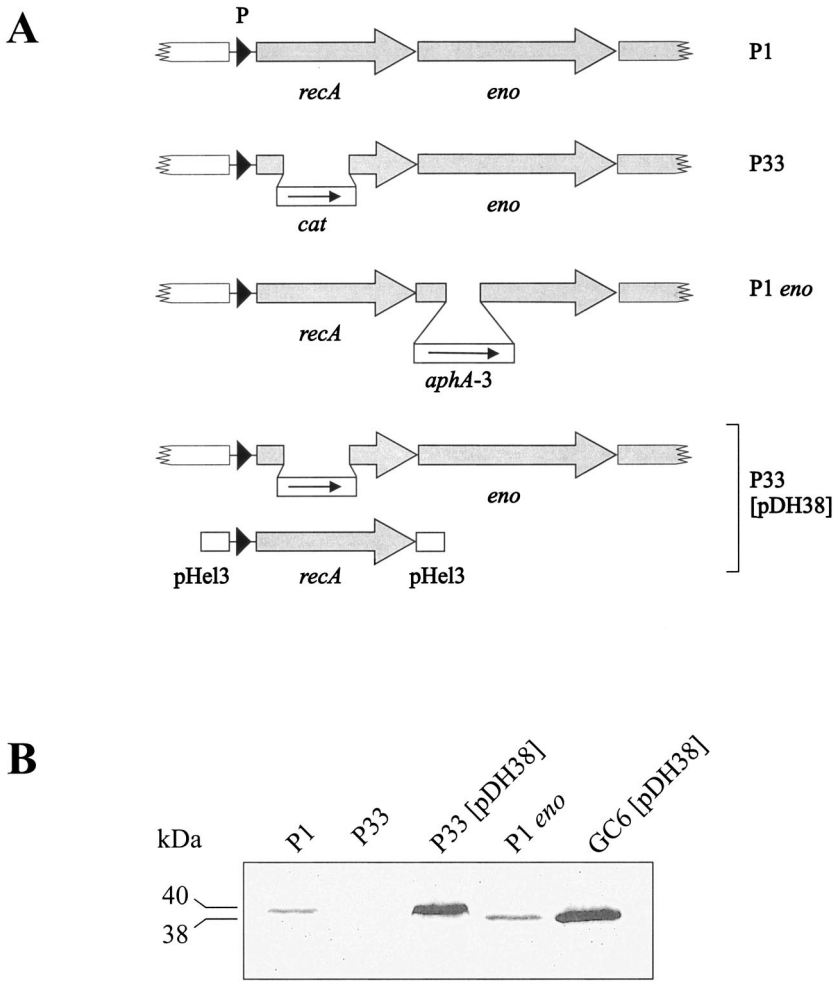

FIG. 1. Construction and complementation of *H. pylori recA* and *eno* mutants. (A) The isogenic *recA* mutant in strain P1 (P33) was constructed by replacing a central part of the *recA* gene by a terminatorless chloramphenicol resistance (*cat*) cassette, and the *eno* mutant was constructed by insertion of an *aphA-3* gene in an internal *Sph*I site. P33 was complemented by using the *recA* gene including its promoter cloned on the shuttle vector pHel3 (pDH38). (B) Total cell lysates of *H. pylori* strains P1, P33, P33(pDH38) and P1 *eno* and of *E. coli* strain GC6(pDH38) were applied to an SDS 6 to 12% gradient gel, blotted, and reacted with antiserum AK263. The size difference between the RecA proteins produced in P33(pDH38) and GC6(pDH38) suggests that RecA is subject to posttranslational modification in *H. pylori.*

protein was obviously unmodified. Since insertion of the kanamycin resistance cassette in the *eno* gene may have a polar effect on the expression of downstream genes such as *hp158*, we sought to check whether the *eno* gene itself is involved in modification. Therefore, we constructed a P1 mutant with a terminatorless chloramphenicol resistance cassette (11) inserted into the *eno* gene. This mutant produced an unmodified RecA protein as well (data not shown), suggesting that both *eno* and *hp158* (and possibly other genes in the locus) are involved in RecA modification.

Glycosylation as a possible modification. The *C. jejuni* general glycosylation locus consists of 10 *pgl* genes (*pglA* to *pglJ*) that have been shown to be involved in protein glycosylation (25, 42), but it also contains the *galE* gene, which is usually associated with lipopolysaccharide (LPS) biosynthesis. Although protein glycosylation is not a common feature of cytoplasmic proteins, we considered the possibility of a RecA glycosylation in *H. pylori*. Genes involved in early steps of LPS biosynthesis may provide the substrates for glycosylation reactions. Therefore, we constructed isogenic *H. pylori* P1 mutants defective in the *galE* gene (*hp360*) encoding a UDP-glucose-4-epimerase, or the *pmi* gene (also called *rfbM* or *hp43*) encoding a bifunctional mannose-6-phosphate isomerase/GDP-mannose pyrophosphorylase, both of which are involved in LPS biosynthesis (8). The *galE* gene of strain P1 was disrupted with plasmid pGH26 (24), and the *pmi* gene was disrupted with plasmid pDHO25::Tn*Max5-3* (18). Both mutations resulted in the production of a RecA protein without an apparent modification (Fig. 3), suggesting that the RecA modification may indeed be a glycosylation. A sequence comparison of RecA proteins from different organisms reveals the presence of an asparagine glycosylation site in the RecA proteins of *H. pylori* and *C. jejuni* but not in the RecA proteins of most other bacteria (Fig. 4A). Although enzymatic deglycosylation of the *H. pylori* RecA protein with *N*-glycosidase F was not successful (data not shown), we wondered whether the putative aspara-

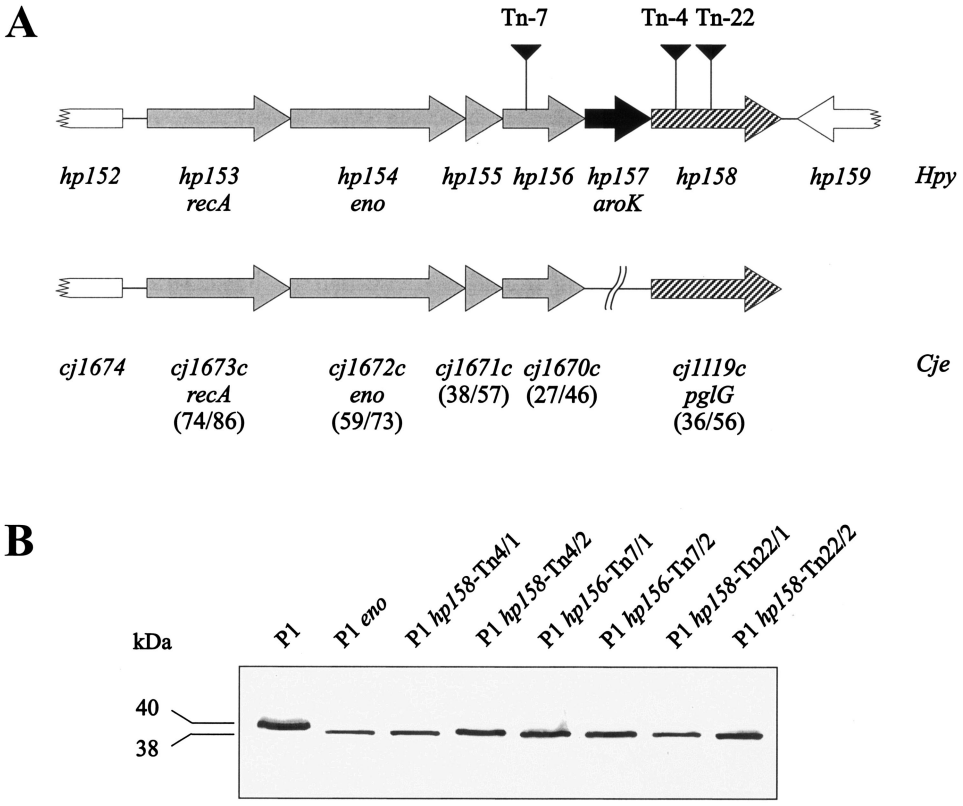

FIG. 2. Presence of a putative operon at the *recA* locus in *H. pylori* and effect of downstream genes on RecA size. (A) Downstream of the enolase gene, there are four further genes which are putatively cotranscribed with *recA* and *eno*. Homologues to the first four genes are arranged identically in the *C. jejuni recA* locus. The last gene of the putative operon displays a significant homology to the *C. jejuni* gene *pglG*, whose product is involved in protein glycosylation. Numbers in parentheses are percents identity/similarity between the *H. pylori* and *C. jejuni* proteins. (B) Tn*Max5* transposon insertions in the genes *hp156* and *hp158* in strain P1 result in the production of unmodified RecA proteins.

gine glycosylation site is important for the RecA modification. Therefore, we decided to introduce a site-specific mutation into the glycosylation site. By a PCR-based mutagenesis procedure, we replaced the codon ACT for the second threonine in the motif with an ATG codon, resulting in a threonine-to-methionine (T189M) mutation. A methionine is present at this position in the RecA protein of *Bordetella pertussis* and thus should be tolerated. The mutated *recA* gene was introduced into *H. pylori* P33 on the pHel3 shuttle vector (pWS127). Western blot analysis of the corresponding *H. pylori* strain demonstrates the overproduction of a RecA protein which indeed seems to be unmodified (Fig. 4B).

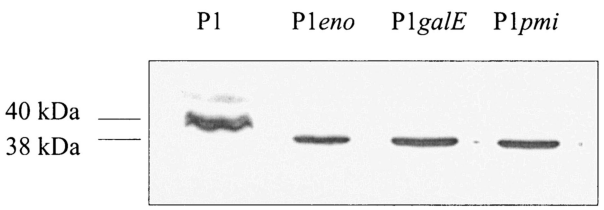

FIG. 3. Effect of mutations in the *galE* and *pmi* genes. Total cell lysates of wild-type strain P1 and the isogenic mutants *eno*, *galE*, and *pmi* were separated by SDS-PAGE, immunoblotted, and reacted with antiserum AK263.

Membrane association of the *H. pylori* RecA protein. One possible function of posttranslational modifications of a cytoplasmic protein might be membrane targeting. Localization of the *H. pylori* RecA protein has not been examined so far, but RecA has been shown to be targeted to the cytoplasmic membrane during transformation in the naturally competent bacteria *Streptococcus pneumoniae* (27). *H. pylori* cells were separated into cytoplasmic and total membrane fractions, and the RecA content of these fractions was determined by immunoblotting (Fig. 5). Considerable amounts of the protein were associated with the total membrane fractions, but RecA was also present in the cytoplasmic fractions. Membrane-bound RecA could be removed by treatment with 1 M NaCl or with 0.1 M sodium carbonate buffer (pH 11), which indicates a peripheral membrane association (data not shown). The distribution between the cytoplasm and the total membrane fraction was, however, independent of the size of the RecA protein, suggesting that the putative modification is not involved in membrane targeting. This is also supported by the RecA (T189M)-producing mutant, which displays the same distribution (data not shown). Interestingly, however, the modification seems to be removed in the cytoplasmic form of RecA during cell fractionation but not in the membrane-bound form. Since we detect only the modified form in a total cell lysate, we would conclude that an activity which removes the modification is

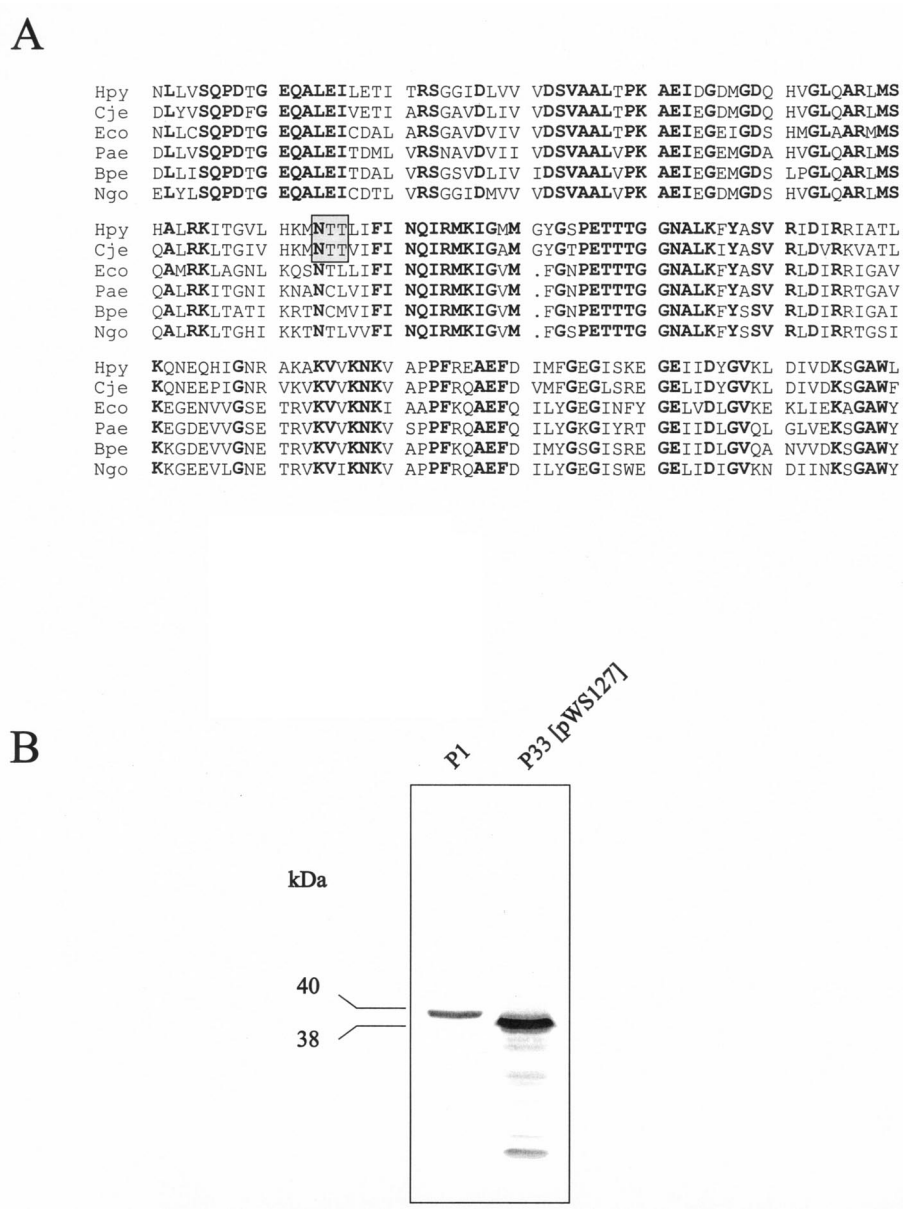

FIG. 4. (A) Sequence comparison of RecA proteins from different bacteria. A putative *N*-glycosylation site (boxed) is present in the RecA proteins of *H. pylori* and *C. jejuni* but not in RecA proteins of other bacteria. *Hpy*, *H. pylori*; *Cje*, *C. jejuni*; *Eco*, *E. coli*; *Pae*, *Pseudomonas aeruginosa*; *Bpe*, *B. pertussis*; *Ngo*, *N. gonorrhoeae*. (B) Size difference between the wild-type RecA protein and the RecA protein with a site-specific mutation (T189M).

released during fractionation which does not affect the membrane-bound form.

Phenotypic effects of RecA modification. Since the enolase mutant displayed a morphology that suggested a cell division defect and since RecA has an effect on cell division in *Bacillus subtilis* (37), we first examined the influence of RecA modification on bacterial cell morphology. Whereas the wild-type strain P1 grown in liquid culture exhibited a curved morphology with a certain degree of clumping (Fig. 6A), the *hp156* (data not shown) and the *hp158* mutants (Fig. 6B) seemed to be shorter and more spiral shaped. An influence on cell division, however, was only visible in the enolase mutant (Fig. 6C), as it displays a considerable number of elongated cells. This

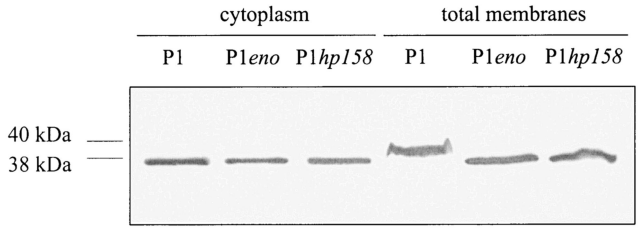

FIG. 5. Association of *H. pylori* RecA with the bacterial membrane. *H. pylori* wild-type strain P1 and the isogenic *eno* and *hp158* mutants were separated into total membrane fractions and cytoplasmic fractions. Immunoblotting with AK263 reveals not only a RecA association with the membranes but also a localization in the cytoplasm. Equal amounts of membrane fractions and cytoplasmic fractions were loaded in each lane.

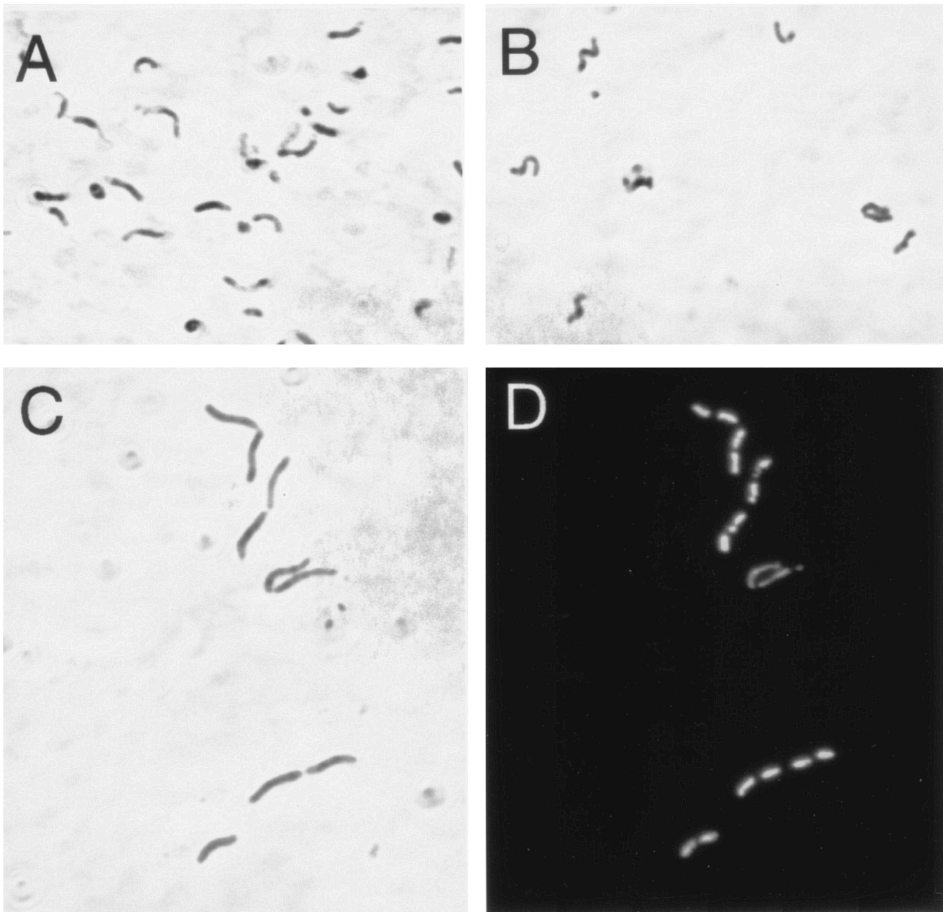

FIG. 6. Effect of mutations in the *recA* locus on bacterial morphology. *H. pylori* wild-type strain P1 (A) and the isogenic mutants P1*hp158* (B) and P1*eno* (C and D) were grown in liquid culture and prepared for microscopy during exponential growth. Panels A to C are phase-contrast micrographs, and panel D shows the same view as in panel C but with DAPI fluorescence.

cell division defect becomes especially apparent after nucleoid staining with DAPI (Fig. 6D). Since only the enolase mutant but not mutants defective in *hp156* or *hp158* display this phenotype, it is probably independent of the RecA modification but dependent on the presence or absence of the enolase.

The *H. pylori* RecA protein is necessary for natural transformation competence (36), for resistance against DNA damage caused by UV radiation, chemical mutagens, or antimicrobial agents such as metronidazole, and for survival at low pH (44). To determine the influence of RecA modification on these phenotypes, we performed transformation competence measurements and UV radiation, low pH, and metronidazole sensitivity assays. Surprisingly, we did not find any difference between the wild-type strain P1, the isogenic mutants in the enolase, *hp156*, or *hp158* genes, and the RecA (T189M)-producing strain with respect to transformation competence, UV, or acid resistance (data not shown). When we compared, however, the sensitivity against metronidazole, the wild-type and mutant bacteria behaved differently (Fig. 7). In comparison to the *recA* mutant, both the *eno* and *hp158* mutants displayed an increased resistance to metronidazole, but it was significantly lower than in the wild type. The metronidazole sensitivity of the *recA* mutant could be complemented with wild-type RecA

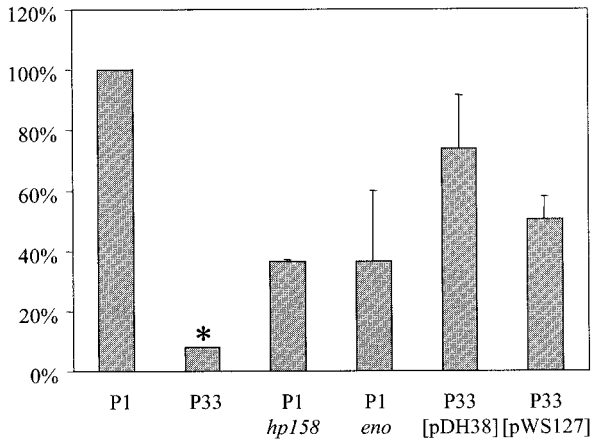

FIG. 7. Influence of RecA modification on metronidazole sensitivity. *H. pylori* wild-type strain P1, the isogenic *recA* (P33), *eno*, and *hp158* mutants, and P33 complemented with wild-type RecA (pDH38) or with RecA (T189M) (pWS127) were assayed for metronidazole sensitivity with an E-test. Due to variations between individual experiments, results were calculated as ratios of MICs for the mutants in comparison to those for the wild-type for each experiment. The MIC for the P1 wild-type strain was on average 0.25 μg/ml. All results are expressed as means of the results from at least three independent experiments. *, The MIC for strain P33 was 0.016 μg/ml in only one of five experiments, otherwise it was <0.016 μg/ml.

produced from the shuttle plasmid pDH38, whereas it was only incompletely complemented with RecA (T189M) produced from plasmid pWS127. These results suggest that the modification is needed for the full function of the RecA protein in *H. pylori*.

DISCUSSION

Posttranslational modification, such as glycosylation, phosphorylation, or acylation, is a common means for modulating structural and functional properties of proteins. In eucaryotic cells, for instance, most of the cell surface and secreted proteins are glycosylated. Such modifications are less abundant in bacteria, but more and more posttranslational modifications of surface-exposed proteins are being described. In particular, the glycosylation of bacterial surface proteins is now recognized as a common posttranslational modification (2, 32). For example, the pilin subunits of *Neisseria meningitidis* (45) and the flagellins of *C. jejuni* (7, 14), *Helicobacter felis* (20), and *H. pylori* (21, 35) are modified by glycosylation. We describe here a putative modification of the *H. pylori* RecA protein, which as a cytoplasmic protein, is more unusual and has not been described for any bacterial RecA protein so far.

The RecA protein is a ubiquitous protein which combines many functions on a relatively short polypeptide chain. In *E. coli*, it has different DNA-binding sites, an ATP-binding site, and coprotease activities for the LexA repressor, the UmuD protein, and various phage repressors. Accordingly, there are various mechanisms involving RecA for repairing damaged DNA. Since a LexA homolog is not present in the *H. pylori* genome and since an SOS response pathway also seems to be absent in *H. pylori*, a coprotease activity may be dispensable for the *H. pylori* RecA protein. However, the RecA protein of *S. pneumoniae*, where a *lexA* homologue is also lacking, is nevertheless able to cleave the *E. coli* LexA protein (38). In *E. coli*, one of the main functions of RecA in response to DNA-damaging agents is to help in repairing stalled replication forks. There are two mechanisms for this situation: error-free replication restarts catalyzed by polymerase II (PolB), probably in conjunction with the restart primosome containing (among other proteins) PriA (34), and error-prone *trans*-lesion synthesis catalyzed by polymerase V ($UmuD'_2C$) (13). Both mechanisms involve RecA for strand invasion during D-loop formation or for formation of an activated nucleoprotein filament (RecA*) ahead of the stalled replication fork, respectively (13). It has been suggested that the role of RecA in these processes is not its recombinational activity but possibly only its DNA-pairing activity (5). *H. pylori* does not seem to possess homologues to either *polB*, *umuC*, or *umuD*, but there are homologues to *priA*, the restart primosome components *dnaB* and *dnaG*, and to the *recR* gene (10), whose product is thought to be involved in loading RecA to single-stranded DNA. It is not clear what the absence of polymerases II and V (and also DinB or polymerase IV) means for the importance of repairing stalled replication forks in *H. pylori*. However, it may be possible that the posttranslational modification of RecA is an adaptation to this different situation. The fact that the absence of the modification has only a slight influence on DNA repair is no contradiction to such a putative role: in *E. coli*, *polB* mutants also do not have any phenotype with respect to UV sensitivity, probably because polymerases II and V can complement each other, although acting at different time points after the induction of damage (30).

One of the functions of RecA-mediated recombination in *H. pylori* is in natural transformation, where incoming DNA has to be recombined into the chromosome. DNA transport across the bacterial membranes is accomplished by a type IV transport system (17), and it may be speculated that there is a direct contact of incoming DNA with membrane-associated RecA. In *S. pneumoniae*, RecA has been shown to be membrane-associated during phases of competence (27). Membrane association in this organism is mediated by the accessory protein CinA (colligrin or competence- and damage-inducible protein). The *cinA* gene is cotranscribed with the *recA* gene, and expression is induced during competent phases. Genes with homology to *cinA* can be found in a number of species, but in many cases, homology extends only to the 3′ region. This is also the case in *H. pylori*, where the *hp952* gene displays a 3′ *cinA* homology. Since the C-terminal part of CinA is probably the RecA-binding domain, this suggests that HP952 would not be able to mediate a membrane association. The RecA protein in *H. pylori* is indeed membrane associated, but this association is not dependent on the posttranslational modification. Our observation that the modification is removed in the cytoplasmic form of RecA but retained in the membrane-associated form, suggests some kind of function for membrane targeting. Defined transformation-competent states or phases have not been described for *H. pylori*, although competence is highest during the early logarithmic phase (19). However, the modification status of RecA does not change during growth phases (data not shown), which suggests that it is not critical for competence.

The *recA* locus in *H. pylori* consists of a putative operon of seemingly unrelated genes, one of which encodes a glycolytic enzyme and another which encodes an enzyme involved in biosynthesis of aromatic amino acids. We show here that at least two of these genes are necessary for RecA modification. It is currently unclear what the contribution of the enolase as a metabolic enzyme might be. Interestingly enough, the closely related organism *C. jejuni* has a *recA* locus with a similar gene arrangement. The *recA* gene is also the first gene of a putative operon, followed by an enolase gene, two genes with homology to *hp155* and *hp156*, respectively, and a putative DNA ligase gene (genes *cj1673c* to *cj1669c*). The product of the *hp156* homolog *cj1670c* has recently been termed *Campylobacter* glycoprotein A (CgpA) due to its lectin-binding activity, and it has been shown to be posttranslationally modified by a glycan containing *N*-acetylgalactosamine residues (25). More recently, the chemical structure of the glycan was determined and shown to be present on at least 21 further *C. jejuni* proteins (47). Since HP156 has a signal sequence, we would predict that it is not involved in the modification process but might rather be a glycosylated surface protein in *H. pylori* as well.

Although we were so far unable to prove this conclusion directly, several lines of evidence suggest that *H. pylori* RecA is posttranslationally modified by glycosylation. (i) It contains a putative asparagine glycosylation motif in contrast to most other RecA proteins. This motif is rather uncommon in bacterial sequences. Of 114 RecA sequences (InterPro entry IPR001553), only 9 contain a putative asparagine glycosylation motif. (ii) Modification is dependent on a gene whose homo-

logue is involved in protein glycosylation in *C. jejuni*. (iii) Site-specific mutagenesis of the glycosylation motif results in production of an unmodified RecA protein, which also has a functional defect. However, a direct detection and molecular characterization of the putative glycosylation remains to be established in further studies.

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