JOURNAL OF BACTERIOLOGY, Dec. 2011, p. 6490–6497 Vol. 193, No. 23
0021-9193/11/$12.00 doi:10.1128/JB.05646-11


# Biochemical and Cellular Characterization of *Helicobacter pylori* RecA, a Protein with High-Level Constitutive Expression▽†

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Received 23 June 2011/Accepted 16 September 2011

***Helicobacter pylori*** **is a bacterial pathogen colonizing half of the world's human population. It has been implicated in a number of gastric diseases, from asymptomatic gastritis to cancer. It is characterized by an amazing genetic variability that results from high mutation rates and efficient DNA homologous recombination and transformation systems. Here, we report the characterization of *H. pylori* RecA (HpRecA), a protein shown to be involved in DNA repair, transformation, and mouse colonization. The biochemical characterization of the purified recombinase reveals activities similar to those of *Escherichia coli* RecA (EcRecA). We show that in *H. pylori*, HpRecA is present in about 80,000 copies per cell during exponential growth and decreases to about 50,000 copies in stationary phase. The amount of HpRecA remains unchanged after induction of DNA lesions, suggesting that HpRecA is always expressed at a high level in order to repair DNA damage or facilitate recombination. We performed HpRecA localization analysis by adding a Flag tag to the protein, revealing two different patterns of localization. During exponential growth, RecA-Flag presents a diffuse pattern, overlapping with the DAPI (4′,6-diamidino-2-phenylindole) staining of DNA, whereas during stationary phase, the protein is present in more defined areas devoid of DAPI staining. These localizations are not affected by inactivation of competence or DNA recombination genes. Neither UV irradiation nor gamma irradiation modified HpRecA localization, suggesting the existence of a constitutive DNA damage adaptation system.**

*Helicobacter pylori*, a microaerophilic Gram-negative bacterium, is the most common etiologic agent of gastric diseases. It colonizes the stomach mucosa of about half of the human population, causing chronic inflammation which can lead to peptic ulcers and, in a small proportion of cases, to adenocarcinoma. *H. pylori* persistence during the whole life of the host and its adaptation to changes within a host or to new hosts are attributed to its extreme genetic diversity. High mutation and recombination frequencies are at the origin of this extreme variability (33). Moreover, *H. pylori* is naturally competent for transformation, horizontal gene transfer between unrelated strains colonizing the same host, thus contributing to its genetic diversity (8, 32). DNA uptake occurs preferentially at cell poles and depends on the type IV secretion system ComB. After processing by nucleases, DNA is internalized through the inner membrane as single-stranded DNA (ssDNA) (30). The ssDNA is then thought to be bound by DprA, which subsequently mediates RecA loading, allowing DNA integration into the recipient chromosome by homologous recombination (HR). DprA was previously shown to be essential for transformation in *H. pylori* (7, 17).

It is clear that HR plays a crucial role in generating the diversity that is characteristic of *H. pylori* (3, 11, 31). HR is also required for genome integrity maintenance, and probably because of this role, it is crucial for colonization of the host (1, 2, 16, 37, 38). Strains lacking intact recombination systems exhibit sensitivity to DNA-damaging agents, hypermutability, and impaired growth rates.

In the two most-studied bacterial models, *Escherichia coli* and *Bacillus subtilis*, two HR initiation pathways coexist (9, 15). The RecBCD (AddAB in *B. subtilis*) pathway is essential for the repair of double-stranded DNA breaks and for resolving regressed forks. The RecFOR pathway is needed for postreplication gap repair and for replication restart after UV damage. It was recently shown that *H. pylori* harbors efficient RecOR and AddAB systems to initiate DNA recombinational repair (1, 17, 18). These ubiquitous initiation pathways metabolize the DNA break to generate single-stranded DNA on which the recombinase RecA is loaded and cooperatively forms a nucleoprotein filament (6). The filament is then aligned with a homologous duplex and promotes strand exchange. The product of this reaction is a branched DNA molecule named the Holliday junction that is processed by the RuvABC complex. Orthologues of these proteins are also present in *H. pylori* and were shown to be important for DNA repair and mouse colonization (12, 16).

RecA is found in all eubacteria, with the exception of a few intracellular symbionts (26). This key recombination enzyme is required for DNA pairing and homologous strand exchange. In *E. coli*, the RecA filament can also activate autocleavage of the LexA repressor to induce the SOS response (21). This coordinated response present in many bacteria induces about 40 genes, including *recA* itself, in response to DNA damage. All the induced genes are involved in DNA repair or lesion tolerance. In *H. pylori*, where SOS seems to be absent, it was recently shown that DNA damage does not induce the expression of DNA repair genes but rather that of genes encoding

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† Supplemental material for this article may be found at http://jb.asm.org/.

▽ Published ahead of print on 23 September 2011.

proteins involved in the uptake of exogenous DNA. Surprisingly, this induction of the transformation machinery is RecA dependent since disruption of *recA* (*hp0153*) prevents induction of competence genes by DNA-damaging agents (7). The mechanism mediating this activation is unknown.

As it is the case for the *E. coli* protein, *H. pylori* RecA (HpRecA) was shown to be necessary for DNA repair. Indeed, *H. pylori recA* mutants are hypersensitive to DNA-damaging agents such as metronidazole or UV or ionizing radiation (18, 25, 35). As expected, intrachromosomal recombination decreases drastically and transformation by an integrative marker is totally abolished in the absence of HpRecA (18, 25). Surprisingly, neither RecOR nor AddAB inactivation reduced transformation efficiencies.

HpRecA has a predicted molecular mass of 37.7 kDa, very similar to that of *E. coli* RecA (EcRecA; 37.8 kDa), with which it shares 58% identity. The protein detected in *H. pylori* cell extracts was shown to have a 40-kDa apparent molecular mass, while EcRecA migrates as a 38-kDa protein, leading to the proposal that in *H. pylori*, HpRecA is posttranslationally modified by glycosylation (10, 25). This glycosylation was shown to be the result of the action of the two genes downstream of *HprecA*. The role of this glycosylation is not yet fully understood since, although it affects metronidazole resistance, UV survival is not impaired in its absence (10). Because the recombinant protein expressed in *E. coli* was insoluble, no biochemical characterization of HpRecA has been reported thus far (10, 25).

Here, we describe the purification of HpRecA and its biochemical characterization. Thus, we conclude that *in vitro*, the *H. pylori* recombinase has activities similar to those of EcRecA. In contrast, we show that expression of HpRecA is not induced following UV or gamma irradiation, but it is constitutive and leads to high levels of the protein in the cells. We also analyzed the cellular localization of HpRecA by following a tagged version of the protein.

## MATERIALS AND METHODS

**Oligonucleotides, enzymes, and reagents.** Oligonucleotides used in this work were from Eurogentec. Their sequences are listed in Table S1 in the supplemental material. Restriction endonucleases, DNA polymerases, and DNA-modifying enzymes were purchased from New England BioLabs (NEB) or Fermentas. EcRecA was from NEB. Culture media and antibiotics used were from AES Chemunex and Sigma-Aldrich, respectively.

**Strains and growth conditions.** All *Helicobacter pylori* strains were in the strain 26695 background (36) and are listed in Table S2 in the supplemental material. Plate cultures were grown at 37°C under microaerophilic conditions (5% $O_2$, 95% $CO_2$, using the MAC-MIC system from AES Chemunex) on blood agar base medium (BAB) supplemented with 10% defibrillated horse blood (AES) and an antibiotics mix. Plates were incubated from 24 h to 5 days depending on the experiment or the mutant selected. Liquid cultures were grown at 37°C with gentle shaking under microaerophilic conditions in brain heart infusion medium (BHI) supplemented with 10% decomplemented fetal bovine serum (Invitrogen).

To generate the corresponding mutant derivatives, the gene of interest cloned into pILL570 was disrupted, leaving the 5′ and 3′ ends (300 bp) of the gene, by a nonpolar cassette carrying either kanamycin (Kn), apramycin (Apr), or chloramphenicol (Cm) resistance genes. DNA was introduced into *H. pylori* strains by natural transformation, and mutants were selected by growth on either 20 μg/ml Kn, 12.5 μg/ml Apr, or 8 μg/ml Cm. Allelic replacement was verified by PCR. Double or triple mutant strains were obtained by plasmid or genomic DNA transformation of the single mutant or by mixing two mutant strains together before plating the mix on double or triple antibiotic selection plates. In the case of multiple mutations, all the disruptions were verified by PCR. At least two independent clones for each construction were studied for each phenotype.

*Escherichia coli* strain BL21(DE3)(pLysS) (Invitrogen) was grown at 37°C in Luria-Bertani medium (LB) in the presence of 34 μg/ml chloramphenicol.

**Construction of RecA-Flag fusion gene.** A fragment containing the last 300 bp of open reading frame (ORF) *hp0153* and the downstream 300 bp was amplified by PCR from the strain 26695 genome with oligonucleotides Ohp153A and Ohp154B as primers. The 622-bp fragment was cloned into pJET1.2 (Fermentas), leading to a plasmid named pPR938. pPR938 was subjected to PCR analysis using oligonucleotides Osf136 and Osf137 to amplify the whole plasmid, which was disrupted before the stop codon of hp0153. The fragment obtained was digested with EcoRI and BamHI. A Flag tag was joined to the nonpolar Kan gene (27) using oligonucleotides Osf172 and Osf173 and cloned into pJET1.2. The fragment of interest was then excised with EcoRI and BamHI and purified. The plasmid and the fragment harboring the Flag tag and the Kan gene were ligated to obtain plasmid pPR943. DNA was introduced into *H. pylori* strains as described above. Genetic and microscopy analyses were performed on two independent clones. Allelic replacement and protein expression were verified by PCR and Western blot analysis, respectively.

**Sensitivity assay.** Bacterial cells were serially diluted, and 10 μl of each dilution was spotted on BAB. For UV-induced DNA damage, cells were irradiated with 0, 15, 30, 45, or 60 J of 264 nm UV light delivering 1 J/$m^2$/s. Gamma irradiation was performed using a $^{137}$Cs source delivering 30 Gy/min. Survival was determined as the number of cells forming colonies on plates after a given irradiation dose divided by the number of colonies from nonirradiated cells.

**Natural transformation assay.** Genomic DNA (200 ng) from strain LR133 (streptomycin [Str] resistant) was mixed with 15 μl of cells resuspended in peptone water ($2.5 \times 10^5$ cells) from exponential- or stationary-phase cultures. Mixes were incubated for 1 h at 37°C, and dilutions of the cells were plated on BAB with and without the appropriate antibiotic (50 μg/ml Str) and incubated for 3 to 5 days. Transformation frequency was calculated as the number of resistant colonies per recipient CFU. *P* values were calculated using the Mann-Whitney U test.

**Cloning, expression, and purification of the recombinant HpRecA protein.** *H. pylori* RecA (HpRecA) was overexpressed in *E. coli* strain BL21(DE3)(pLysS) (Invitrogen) as an N-terminal maltose-binding protein (MBP)-tagged protein. For this purpose, ORF hp0153 was amplified from *H. pylori* 26695 genomic DNA using the Osf107 and Osf158 primers and the amplification product was cloned into pMALc2x (NEB). *E. coli* BL21(pLysS) cells transformed with pMALc2x-*HprecA* were grown in LB plus 34 μg/ml chloramphenicol, 100 μg/ml ampicillin, and 2 g/liter glucose at 37°C to an optical density at 600 nm ($OD_{600}$) of 0.4. Isopropyl-β-D-thio-galactopyranoside (IPTG) was added to a final concentration of 0.3 mM. After incubation for 2 h at 37°C, cells were harvested, resuspended in lysis buffer (50 mM Tris [pH 8], 200 mM NaCl, 1 mM dithiothreitol [DTT], 1 mM EDTA, 0.5 mg/ml lysozyme), and disrupted by sonication. After centrifugation at 100,000 × *g* for 20 min at 4°C, the soluble fraction was diluted to 150 mM NaCl and applied on a QMA anion-exchange column. The QMA flow-through was loaded on an amylose column (NEB) equilibrated with lysis buffer. HpRecA-MBP was eluted in lysis buffer containing 10 mM maltose. The MBP-HpRecA pooled protein was treated with 2.5% factor Xa protease (NEB) for 3.5 h at 30°C, resulting in the cleavage of the fusion protein to generate two products, MBP (42 kDa) and HpRecA (38 kDa). The products of proteolysis were desalted on a PD10 column (GE Healthcare) in buffer A (25 mM phosphate [pH 6], 50 mM NaCl, 1 mM EDTA, 1 mM DTT) and loaded on a HiTrap heparin column (GE Healthcare) equilibrated with buffer A. Elution was performed with a 50% gradient of buffer B (25 mM phosphate [pH 6], 2 M NaCl, 1 mM EDTA, 1 mM DTT). Fractions containing HpRecA were concentrated by Amicon (Millipore) filtration and pooled. The recombinant protein was conserved in 50% glycerol.

**ATP hydrolysis assay.** ATPase activity was measured by linking ATP hydrolysis to the oxidation of NADH as described previously (23). Assays were performed at 37°C in buffer containing 50 mM phosphate (pH 6), 750 μM phosphoenolpyruvate trisodium, and 5 mM $MgCl_2$ in the presence of 330 ng ϕX174 single-stranded DNA (10 μM nucleotides; NEB), 0.8 mM NADH, and 0.6 μM HpRecA. Reactions were started by the addition of ATP at the indicated concentrations. Values for the Michaelis-Menten constants $k_{cat}$ and $K_m$ for ATP at saturating amounts of ssDNA were derived by fitting data directly to the Michaelis-Menten equation.

**DNA strand exchange reaction.** M13CEF3 ssDNA (4.5 μM nucleotides; NEB) was incubated in 30 mM phosphate buffer (pH 6), 9 mM $MgCl_2$, 1.8 mM DTT, 1.1 mM ATP, 7.2 mM phosphocreatine, and 9 U/ml phosphocreatine kinase for 3 min at 37°C before the addition of 0.26 μM EcSsb and variable amounts of HpRecA protein. The reaction mixtures were kept at 37°C for 10 min. Then

linearized M13CEF3 double-stranded DNA (dsDNA) (4.5 μM nucleotides) was added. After 90 min of incubation, the reaction mixture was deproteinized and analyzed by electrophoresis in 0.8% agarose gel in Tris-acetate-EDTA (TAE) buffer. The gels were stained with SYBR gold (Molecular Probes) and quantified with ImageJ software.

**Western blot analysis of HpRecA or EcRecA.** Proteins from total cell extracts were separated by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were incubated in blocking buffer (1× Tris-buffered saline [TBS] and 0.1% Tween 20 with 5% nonfat dry milk) for 1 h at room temperature. Incubations with the primary antibodies against either EcRecA (AB63797 from Abcam) or HpRecA (AK263 provided by R. Haas [10]) were carried out for 1 h at room temperature, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary antibody under the same conditions. Proteins were revealed by adding ECL Plus Western blotting detection reagents (GE Healthcare) and by exposure to X-ray film (CL-XPosure film; Pierce). The intensities of the bands were quantified using ImageJ software. To estimate RecA protein amounts before and after irradiation in *H. pylori* or *E. coli*, liquid cultures in exponential or stationary growth phase were irradiated at 10 J or at 75 Gy. Samples were collected before and at different times after irradiation and analyzed by Western blotting. Quantifications were performed with at least three independent experiments.

**Microscopy assays.** Samples from liquid cultures were collected, and the cells were washed once with phosphate-buffered saline (PBS). Cell membranes were stained with FM64-X (Invitrogen) at a 1:500 dilution in PBS for 10 min and washed with PBS. Samples were then spotted on coated gelatin glass coverslips and fixed for 1 h with 4% formaldehyde. Slides were washed four times with PBS containing 0.1% glycine and once with PBS and incubated 1 h at 37°C with mouse anti-Flag antibody (Sigma-Aldrich) at 0.1 μg/ml in blocking solution (PBS, 0.1% Tween, 1% normal goat serum, 3% bovine serum albumin [BSA]). Cells were then washed three times (10 min each) with PBS and incubated with Alexa-Fluor 488 goat anti-mouse IgG antibodies (Molecular Probes) at a 1:1,000 dilution in blocking solution for 1 h at 37°C. DNA was stained with 1 μg/ml 4′,6-diamidino-2-phenylindole (DAPI). Coverslips were mounted in Dako fluorescent mounting medium. Image acquisition was performed with a Leica SPE confocal microscope (Wetzlar, Germany) using an ACS APO 63.0×, 1.30 oil lense. Image treatment and analysis were performed using Leica and ImageJ software programs. Pearson's coefficients were calculated with the JACoP version 2.0 plug-in from ImageJ. The results are the median of at least 3 fields and 2 independent experiments corresponding to more than 500 bacteria. Standard deviations were calculated.

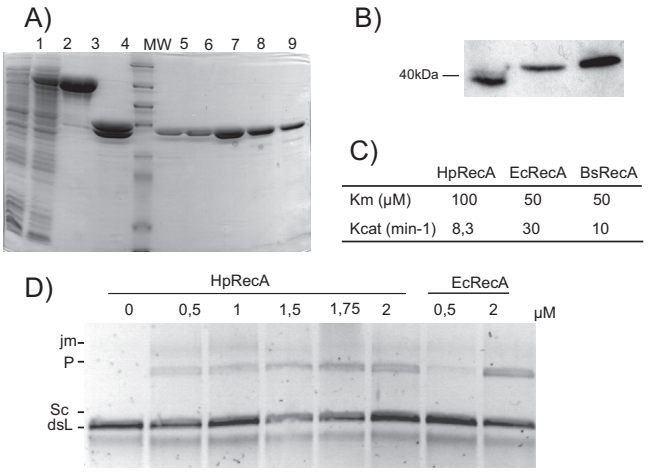


FIG. 1. HpRecA *in vitro* characterization. (A) Recombinant HpRecA expression and purification. SDS-PAGE of purification fractions. Lane 1, uninduced cells; lane 2, induced cells; lane 3, RecA-MBP pool after amylose elution; lane 4, products of factor Xa cleavage; lanes 5 to 9, different HpRecA fractions from heparin column. (B) Size comparison between HpRecA and EcRecA. RecA Western blot analysis was performed with antibodies against EcRecA as follows: total *E. coli* cell extract (lane 1), total 26695 cell extract (lane 2), purified HpRecA (lane 3). (C) Catalytic constants for ATP hydrolysis of HpRecA, EcRecA (19), and BsRecA (29). Assays were performed as described in Materials and Methods. (D) DNA three-strand exchange reactions promoted by HpRecA. Assays were performed as described in Materials and Methods, with HpRecA at the indicated concentrations. The reactions were analyzed by electrophoresis on an 0.8% agarose gel in TAE running buffer. dsL, linear double-stranded DNA; Sc, circular single-stranded DNA; jm, partially strand-exchanged joint molecules; P, fully strand-exchanged nicked circular dsDNA.

## RESULTS

**Expression and purification of recombinant HpRecA.** In order to determine the biochemical properties of the HpRecA protein, we attempted the purification of the protein. His-tagged RecA was previously reported as an insoluble protein found in inclusion bodies when expressed in *E. coli* (10). By expressing HpRecA from 26695 with the maltose-binding protein (MBP) fused to its N terminus, we obtained a soluble protein (Fig. 1A). The fusion protein was then cleaved, and the recombinant HpRecA was purified to near homogeneity as described in Materials and Methods. No nuclease activity was found associated with the purified protein (data not shown).

As mentioned above, it was reported that HpRecA was glycosylated when expressed in the *H. pylori* P1 strain but not in *E. coli* (10). Surprisingly, in our experiments, Western blot analysis of the RecA proteins revealed that both the recombinant and endogenous proteins from the *H. pylori* 26695 strain have an apparent size of 40 kDa, whereas EcRecA migrates as a 38-kDa protein (Fig. 1B), suggesting that in the 26695 genetic background, HpRecA is not modified. Moreover, no changes of the recombinant protein were observed after incubation with the *N*-glycosylase PNGase F (data not shown).

**HpRecA enzymatic activities are similar to those of EcRecA.** ATP hydrolysis by the purified HpRecA was monitored using a coupled spectrophotometric assay. The catalytic constants were determined as described in Materials and Methods. Despite the similarities between HpRecA and EcRecA, HpRecA seems to be less efficient *in vitro* than its homolog, since its kinetic constants are reduced 2- to 4-fold (Fig. 1C) (19). Nevertheless, the constants are in the same range as those of *Bacillus subtilis* RecA (BsRecA) (29). RecA proteins are recombinases, which are able to promote strand exchange reactions between linear double-stranded DNA and homologous circular single-stranded DNA to yield a nicked circular product. When such an activity was tested on HpRecA, as expected, we confirmed that the *H. pylori* recombinase was capable of mediating DNA strand exchange *in vitro* (Fig. 1D).

**HpRecA is an abundant and constitutively expressed protein in *H. pylori*.** In *E. coli* under normal growth conditions, the amount of EcRecA is known to be between 7,000 and 10,000 monomers per cell, increasing up to 70,000 copies after SOS induction (6, 28). Western blot quantification of HpRecA allowed us to estimate that HpRecA is present at about 80,000 monomers per cell during exponential growth and about 50,000 in stationary phase (Fig. 2B, compare lanes E1 and E2 to lanes S1 and S2). This result shows that HpRecA is expressed in *H. pylori* at a level similar to the SOS-induced level in bacteria harboring this response system.

In order to explore whether the cellular levels of HpRecA are modified after a genotoxic insult, we exposed *H. pylori* to UV (10 J/m$^2$) or gamma (75 Gy) irradiation and quantified the amount of HpRecA after 3 h, the time of one generation (Fig.

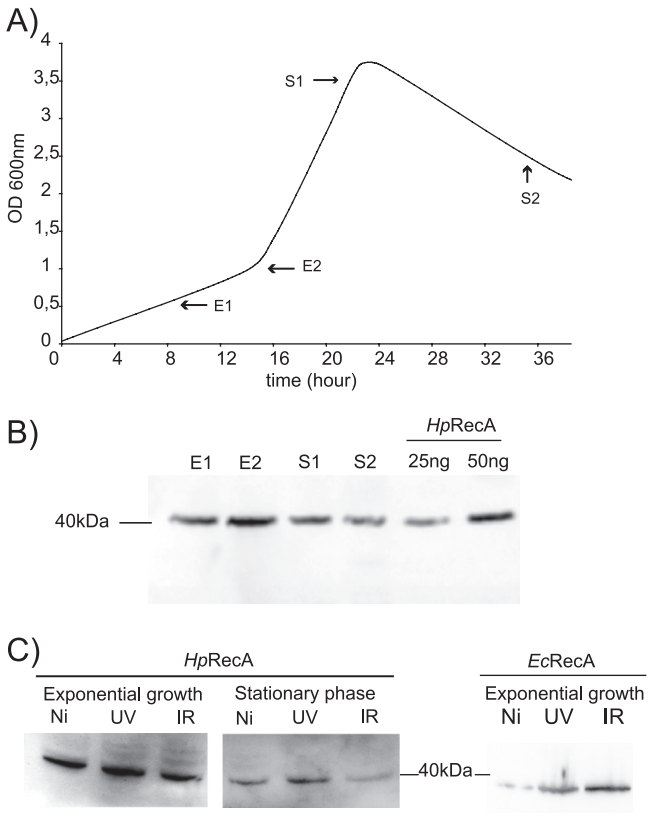


FIG. 2. HpRecA expression analysis. (A) *H. pylori* 26695 strain growth curve. Black arrows indicate when samples were collected. E1, 0.5 OD; E2, 1 OD; S1, 3.5 OD; S2, 2.5 OD. (B and C) Western blot analysis of HpRecA expression. Total cell lysates were separated by SDS-PAGE and immunoblotted with AK263. Equal amounts of cells were loaded in each lane. (B) Quantification of HpRecA during growth phases. For quantification controls, we used 25 ng and 50 ng of purified HpRecA. (C) RecA expression before and after irradiations. Exponential- or stationary-phase liquid cultures were irradiated at 10 J or 75 Gy. *E. coli* samples were collected after 1 h of incubation. *H. pylori* samples were collected after 3 h of incubation. Ni, nonirradiated; UV, ultraviolet irradiation; IR, ionizing irradiation.

2C). No changes in HpRecA levels were observed after induction of DNA damage. We confirmed the induction of EcRecA in *E. coli* after one generation (Fig. 3C) and estimated that the amount of protein per cell increased from around 5,000 to about 50,000 monomers per cell after UV and to about 80,000 after gamma irradiation, confirming previously published data (28). In *H. pylori*, we tested shorter and longer times of recovery after irradiation, as well as exposures of up to 60 J/m$^2$ and 300 Gy without detecting differences in the levels of HpRecA (data not shown). Therefore, we conclude that HpRecA is constitutively expressed at high levels and its expression is not influenced by DNA damage.

**HpRecA is relocalized during stationary phase.** In order to follow the localization of HpRecA in the cell, we expressed a C-terminal fusion of HpRecA to the green fluorescent protein (GFP) from the endogenous *HprecA* locus. We analyzed four independent clones, and all four of them presented Δ*recA* phenotypes, probably due to GFP interference with HpRecA functions (data not shown). We therefore decided to use another tagging system to follow the localization of the protein and fused the 3′ end of the endogenous *HprecA* gene to the sequence encoding the Flag peptide. Three independent clones were analyzed for survival to UV and ionizing radiation. As shown in Fig. 3A and B, the mutants presented a wild-type (wt) phenotype, indicating that addition of a Flag tag to HpRecA did not perturb its ability to repair lesions caused by UV or ionizing radiation. Moreover, the transformation efficiencies of these strains remained unmodified (Fig. 3C) compared to that of the wild type, confirming the functionality of the tagged protein. Western blot analyses using antibodies against RecA showed that the protein level was not modified by the addition of the Flag tag (data not shown).

RecA-Flag was then followed by confocal microscopy using immunofluorescence. As expected from the high-level expression of HpRecA (see above), the protein was easily detectable (Fig. 4). No immunofluorescence could be detected in strains devoid of Flag sequence (data not shown), confirming the specificity of the signal. Images issued from samples collected from exponential-phase cultures (Fig. 4A, expo) presented a diffuse pattern of RecA-Flag that coincided with the DAPI staining pattern corresponding to DNA. When images were obtained from stationary-phase cultures (after 30 h) (see Fig. 2A), the RecA-Flag signal was weaker, confirming the lower levels of protein observed using Western blots (Fig. 2B). RecA-Flag was more concentrated in large foci mostly in mid-cell or, less often, at the poles, corresponding to areas with essentially no DAPI staining (Fig. 4A, stat). To rule out exclusion of DAPI staining by the formation of RecA foci, we reversed the order of the staining protocol. Even when DNA was stained before the antibody was applied to the samples, the foci were devoid of the DAPI signal, strongly suggesting the accumulation of HpRecA in DNA-poor regions. The difference in localization between exponential and stationary phases was confirmed by analyses using ImageJ software and Pearson's coefficient method for quantifying the degree of colocalization between DNA and HpRecA. Results are presented in Fig. 5. By computer analysis of more than 500 cells in each case, we confirmed a better colocalization of RecA-Flag with DAPI in exponential-phase cells. Occasionally, some *H. pylori* bacteria from stationary-phase cultures presented a circular shape corresponding to coccoids, a physiological form known to appear under starvation conditions. RecA-Flag was also detected in these cells (Fig. 4).

**HpRecA localization is not affected by disruption of *dprA*, *comB6*, or recombination mediator genes.** As mentioned in the introduction, during DNA strand break repair by HR, RecA can be loaded onto ssDNA by two different pathways, RecBCD (or AddAB in *B. subtilis* and *H. pylori*) or RecFOR (or RecOR in *H. pylori*). We replaced the *HprecA* gene with the RecA-Flag gene in *H. pylori* strains deleted for either *recO* or *addA* or both. Western blot analysis using antibodies against RecA confirmed that the level of RecA-Flag expression is not modified by the inactivation of the recombination genes studied (data not shown). Analysis of RecA-Flag localization (Fig. 5; see also Fig. S1 in the supplemental material) showed that the RecA-Flag localization pattern in the mutants is identical to that found in the wild-type strain. This was true for both the exponential-phase culture (a diffuse pattern) and the stationary-phase culture (formation of discrete foci).

The concentration of RecA-Flag at the pole of some cells in

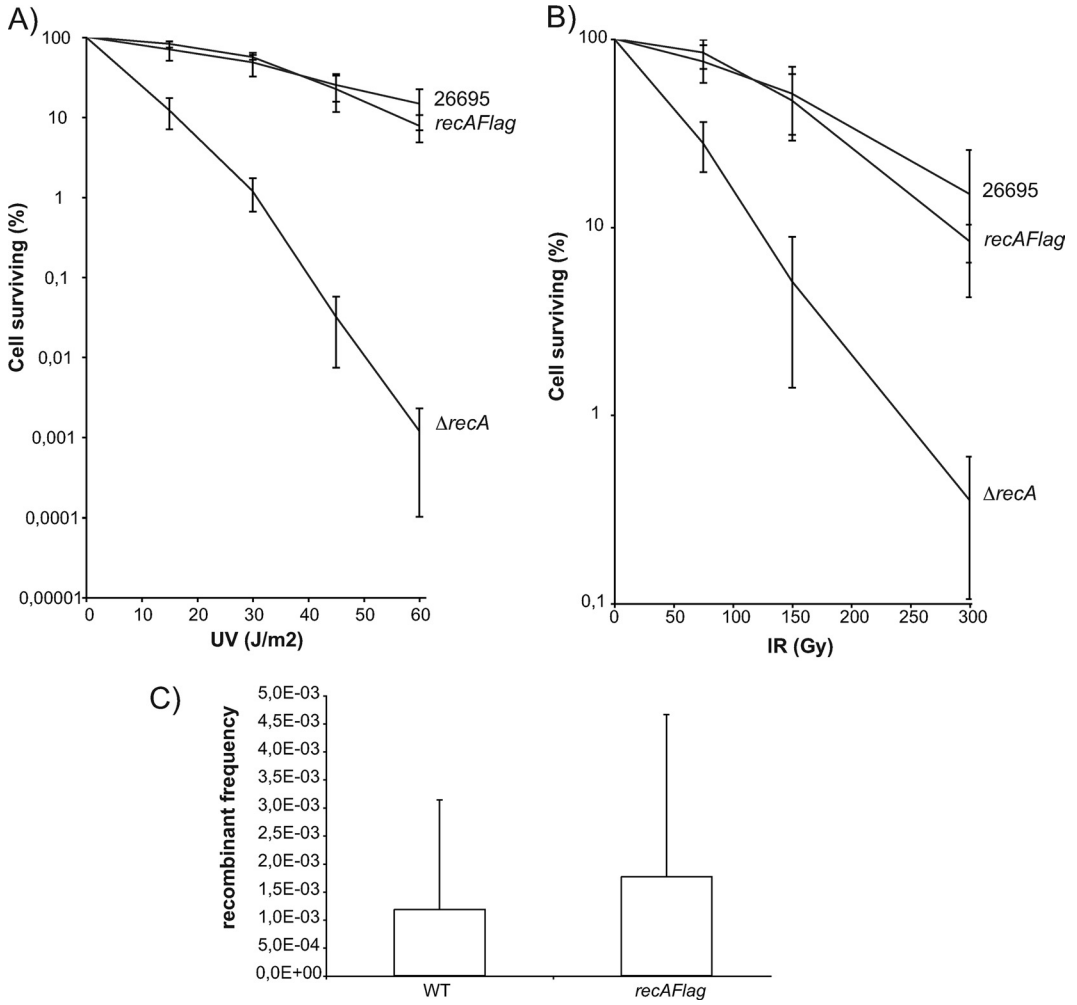


FIG. 3. Analysis of strains harboring the RecA-Flag gene instead of *recA*. (A) UV irradiation sensitivities; (B) gamma irradiation sensitivities. Irradiations were performed as described in Materials and Methods. Averages from three experiments are shown. (C) Transformation frequencies.

stationary phase evoked the relocalization of BsRecA in *Bacillus subtilis* cells where competence is induced (14). To test whether the pattern of relocalization of HpRecA described for stationary-phase cultures was linked to DNA uptake, we disrupted either *dprA* or *comB6* genes in the strain expressing RecA-Flag. DprA is the mediator protein loading RecA on transforming DNA, and ComB6 is part of the membrane ComB complex responsible for transforming DNA entrance into the cell. In both cases, the transformation capacity was completely abolished (data not shown). Analysis of RecA-Flag localization in both mutant strains revealed that RecA-Flag relocalization during stationary phase is not altered (Fig. 4B and C). Computer analysis of images confirmed these observations (Fig. 5). Therefore, localization of RecA-Flag at the pole of some cells during stationary phase seems to be independent of the transformation process.

**HpRecA distribution is unchanged after UV or gamma irradiation.** EcRecA relocalization after DNA damage formation was described in *E. coli* by following the expression of EcRecA fused to GFP from the endogenous promoter (24). Both the SOS-dependent induction of its expression and the relocalization into foci were observed after a genotoxic stress. In *B. subtilis*, BsRecA fused to GFP and expressed from a strong promoter was also shown to relocalize in response to mitomycin C treatment (13). We showed above that HpRecA expression is not modified by DNA damage. We wanted to test whether HpRecA changes its distribution pattern after exposure of the cells to UV or gamma irradiation. Liquid cultures in exponential growth or in stationary phase were submitted to irradiation at various doses and allowed to recover under normal growth conditions. Samples were taken at different times and analyzed. Between 15 min and 30 h after irradiation with up to 60 J/m$^2$ of UV or 300 Gy of ionizing radiation, we were not able to see any relocalization of the protein (Fig. 6). This absence of relocalization after DNA damage was also observed in the recombination mutants studied (see Fig. S1 in the supplemental material).

## DISCUSSION

We present here the biochemical and cellular characterization of the HpRecA protein. The purification of a recombinant

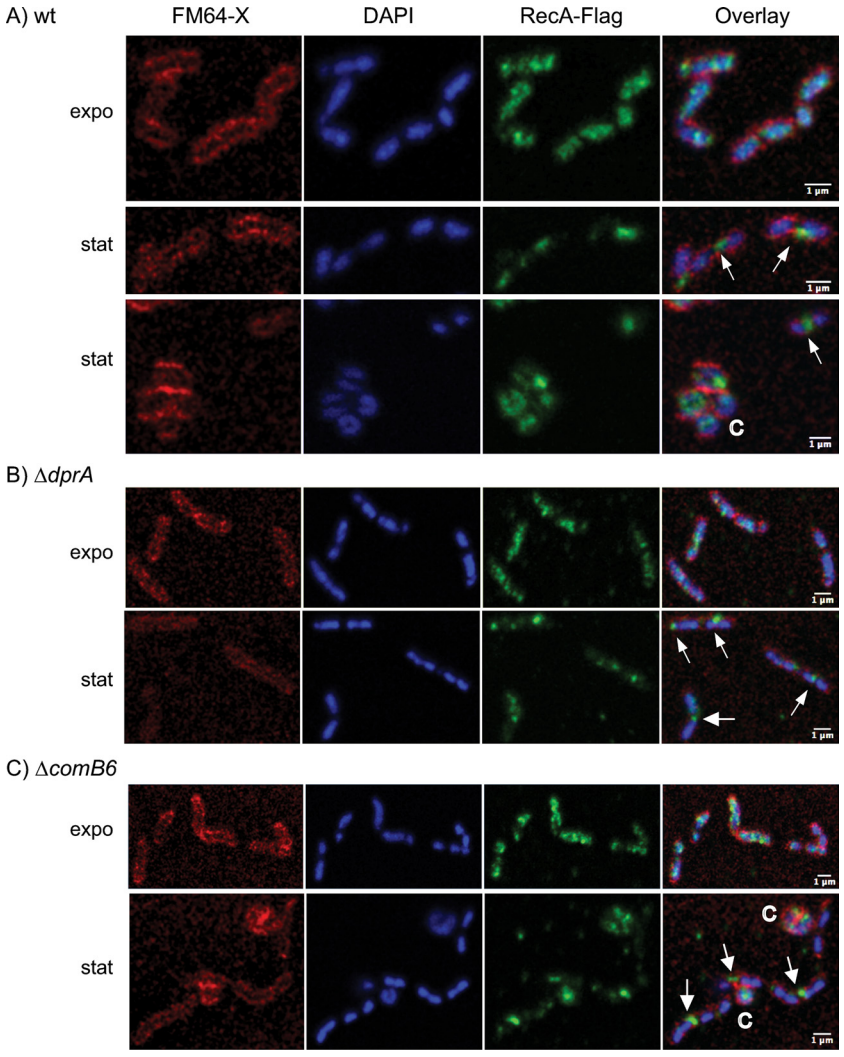


FIG. 4. Localization of RecA-Flag in *Helicobacter pylori*. A Flag tag was fused to HpRecA, and the fusion protein was expressed at the *HprecA* locus. Images were obtained with a confocal microscope as described in Materials and Methods. (FM64-X and DAPI are membrane and DNA stainings, respectively.). Samples were obtained from liquid cultures in exponential (expo) or stationary (stat) phases. Coccoid forms are indicated by the letter C. White arrows indicate RecA-Flag foci excluded from DNA. RecA-Flag in 26695 wild-type (A), Δ*dprA* (B), and Δ*comB6* (C) strains.

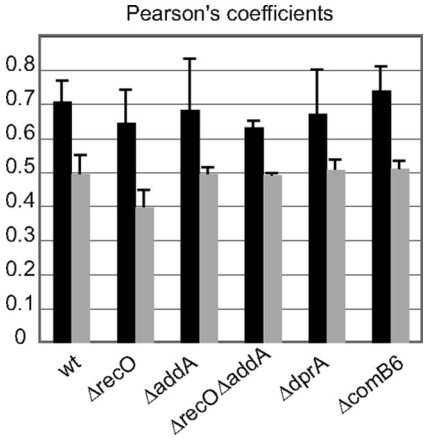


FIG. 5. RecA-Flag localization analysis. Pearson's coefficient between DNA (DAPI) and RecA-Flag were determined as described in Materials and Methods. Black bars correspond to exponential phase. Gray bars correspond to stationary phase. The different mutant strains analyzed are indicated.

protein allowed the first analyses of its enzymatic properties. The determination of the kinetics parameters revealed ATPase and recombinase activities for the recombinant protein similar to but somewhat lower than those of EcRecA and BsRecA (Fig. 1). It was first reported that HpRecA is modified *in vivo* by glycosylation, leading to a molecular mass increase of 2 kDa (25). Our comparison of the purified HpRecA with EcRecA confirmed the size difference. However, recombinant HpRecA expressed in *E. coli* migrated as the endogenous HpRecA (Fig. 1B), suggesting that either the protein expressed in *E. coli* is modified as in *H. pylori* or that it simply displays an aberrant migration in PAGE. The genes involved in HpRecA glycosylation (*hp0156* and *hp0158* [10]) do not have homologues in *E. coli*, but the presence of functional analogues that may modify the ectopically expressed HpRecA may not be excluded *a priori*. The smaller size reported by Schmitt et al. (25) for the recombinant HpRecA expressed in *E. coli* may in that case be explained by the fact that the protein was insoluble and there-

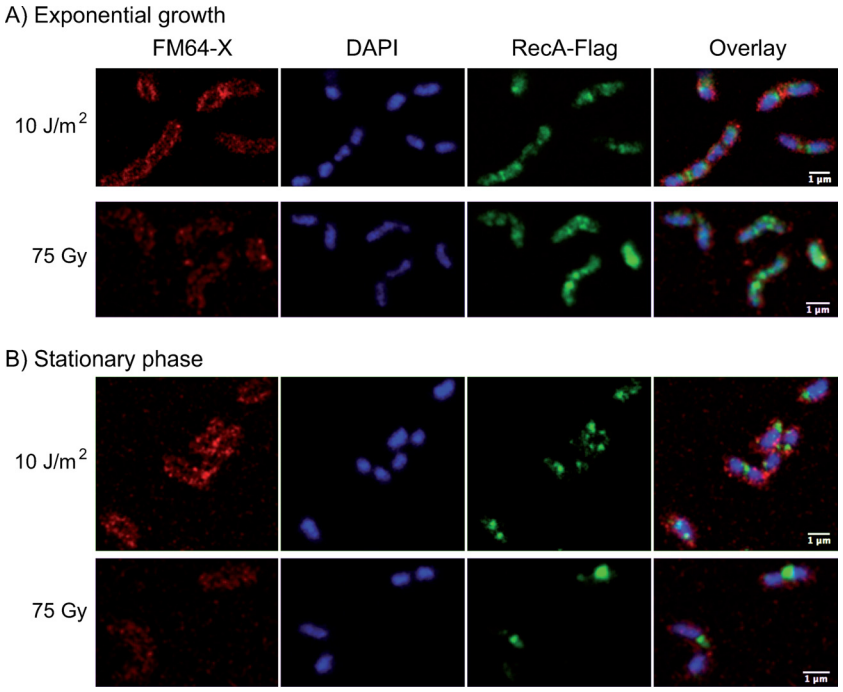


FIG. 6. Localization of RecA-Flag after UV (10 J/m$^2$) or gamma (75 Gy) irradiation. See the Fig. 4 legend for details. Samples were collected 4 h after irradiation of liquid cultures in exponential (A) or stationary (B) phases.

fore not recognized by the glycosylation machinery. However, the absence of apparent variation in molecular size after a glycosylase treatment of the *E. coli*-expressed HpRecA strongly suggests that in the 26695 background, the bulk of the protein is not modified and that the higher apparent molecular size is due to a migration aberration.

Although HpRecA enzymatic activities do not show significant differences with those of other well-characterized RecA proteins, some striking differences were revealed by the determination of the HpRecA protein expression pattern in *H. pylori* cells.

First, we showed that the protein is present in about 80,000 copies per cell in exponential-phase cultures, a level similar to that of EcRecA in SOS-induced bacteria (Fig. 2). We observed that the number of HpRecA molecules per cell decreases to 50,000 copies during stationary phase (Fig. 2). This result is consistent with the reported 2-fold decrease of *HprecA* transcripts in stationary cultures (7). This high expression, together with the predicted absence of an SOS system in *H. pylori*, suggests that HpRecA is always highly expressed to protect chromosome integrity. This hypothesis may be related to *H. pylori* infectivity. Indeed, it was reported that strains disrupted for the *HprecA* gene are impaired in mouse colonization capacity (1). Recombination is therefore essential for colonization and probably also for survival in the host, where bacteria are constantly subjected to the inflammatory response of the host (22). Consistently, induction of DNA damage does not increase the expression of DNA repair genes, including *HprecA*, but rather that of genes coding for the competence machinery components (7). The induction of competence by genotoxic agents was previously reported for other pathogens, such as *Streptococcus pneumoniae* (5), *Coxiella burnetii* (20), and *Legionella pneumophila* (4). Induction of competence genes instead of the SOS system, absent in these organisms, may then be an adaptation to pathogenicity.

Since a critical function of RecA proteins is the rescue of stalled or collapsed replication forks, it can be expected that higher levels of the protein are required during exponential growth. The lower levels of HpRecA observed in stationary phase can then be correlated with the reduced need for RecA in nonreplicating cells.

Furthermore, the use of a tagged version of the protein (RecA-Flag) that is fully active (Fig. 3) allowed us to follow the localization of the protein in cells. We showed that RecA-Flag colocalizes with DAPI-stained DNA during exponential growth. This pattern is modified during stationary phase when the protein is present in one to three foci per cell, preferentially in areas excluded from DAPI staining (Fig. 4A and 5). This change can be related both to the reduced amount of HpRecA and to the absence of replication. RecA-Flag is found on DNA in exponential-phase cultures when replication is active. It may not be needed there once DNA synthesis is complete. On the other hand, the relocalization may also be explained by a competence signal that leads to HpRecA migration to the membrane and the DNA uptake machinery (10). However, the same pattern of RecA-Flag in wild-type cells was observed in both Δ*dprA* and Δ*comB6* strains, indicating that the relocalization during stationary phase is not dependent on these proteins. Even if RecA-Flag relocalization is due to a competence signal, it is not linked to the entrance or formation of a DprA filament on the entering DNA.

Perhaps more striking is the absence of RecA-Flag relocalization after induction of DNA damage (Fig. 6). We tested UV and gamma radiations, and neither of them induces changes in

RecA-Flag localization pattern. This absence of redistribution may be due to the large amount of HpRecA present in the cell at all times, even in the absence of DNA damage, in contrast to what occurs in *E. coli* or *B. subtilis*. It can be proposed that the protein is always present in sufficient quantity to ensure the repair of chromosomal DNA, thus avoiding the requirement for a specific recruitment to the damaged region. The RecA-Flag localization is also independent of the HR initiators RecO and AddA. Localization is indeed not affected in nonirradiated Δ*recO*, Δ*addA*, or Δ*recA* Δ*addA* mutants (Fig. 5). Moreover, after different times of recovery and irradiation doses, no modifications were observed in HpRecA localization (see Fig. S1 in the supplemental material).

Interestingly, *H. pylori* does not seem to be particularly resistant to DNA damage, compared to *E. coli* and *B. subtilis*, but presents a high spontaneous mutation rate and an efficient transformation system. In response to modifications of environmental conditions, mutations can appear and spread among the entire infecting population by transformation and recombination, explaining the panmictic characteristics of *H. pylori* populations (8, 34). The constant presence of a large amount of available RecA in the cell may contribute to the adaptation capacity of this pathogen.

## ACKNOWLEDGMENTS

We thank Rainer Haas for his gift of antibodies AK263 against HpRecA. We thank T. Kortulewski from the IRCM Microscopy Facility for technical assistance with microscopy analyses. We thank P. Servant for suggestions regarding the manuscript.

This work was supported by a grant from the Agence Nationale de la Recherche (ANR-09-BLAN-0271-01 to J.P.R.), the CNRS, and predoctoral fellowships from the CEA and the Fondation pour la Recherche Medicale (to E. Orillard).

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