DprB Facilitates Inter- and Intragenomic Recombination in Helicobacter pylori

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For naturally competent microorganisms, such as Helicobacter pylori, the steps that permit recombination of exogenous DNA are not fully understood. Immediately downstream of an H. pylori gene (dprA) that facilitates high-frequency natural transformation is HP0334 (dprB), annotated to be a putative Holliday junction resolvase (HJR). We showed that the HP0334 (dprB) gene product facilitates high-frequency natural transformation. We determined the physiologic roles of DprB by genetic analyses. DprB controls in vitro growth, survival after exposure to UV or fluoroquinolones, and intragenomic recombination. dprB ruvC double deletion dramatically decreases both homologous and homologous transformation and survival after exposure to DNA-damaging agents. Moreover, the DprB protein binds to synthetic Holliday junction structures rather than double-stranded or single-stranded DNA. These results demonstrate that the dprB product plays important roles affecting inter- and intragenomic recombination. We provide evidence that the two putative H. pylori HJRs (DprB and RuvC) have overlapping but distinct functions involving intergenomic (primarily DprB) and intragenomic (primarily RuvC) recombination.

Genetic diversity, a characteristic of many bacterial populations, maximizes survival when ecological niches change and reflects the major impact of horizontal gene transfer (18, 69). The Gram-negative slow-growing bacterium Helicobacter pylori, which colonizes the human stomach and resists gastric inflammation and whose persistence affects the risk of gastrointestinal diseases (10), is naturally competent for DNA uptake. H. pylori isolates from individual hosts have remarkable genetic diversity, reflecting adaptation to their gastric habitats and to inflammation (2, 29, 43, 46, 50, 66); their ability to be transformed contributes to the development of variation (12, 26, 28, 39), and recombination involves multiple mechanisms (31, 73).

Natural transformation of bacterial cells involves three steps: exogenous DNA binding, uptake, and translocation, and recombination with genomic DNA (15). The early steps of H. pylori natural transformation require comB genes that encode homologs of a type IV secretion system facilitating DNA uptake (26). A DprA-family protein encoded by HP0333 plays roles in high-frequency uptake and translocation of exogenous DNA (4, 62, 63), and RecA (HP0153) is required for the homologous recombination inherent in H. pylori transformation (55, 70). Expression of H. pylori natural competence genes can be induced by DNA damage, further increasing transformation frequency (TF) and gene exchange (20).

In H. pylori, natural transformation is suppressed by the DNA helicases RuvB and RecG (30, 32). Holliday junctions (HJs), four-way branched DNA intermediates produced at the last step of homologous recombination, are recognized by Holliday junction resolvases (HJRs), specialized DNA-binding proteins that are required to complete recombination (59). HJ structures that are typically formed by the crossover of parental and invading strands of DNA during recombination (42) are migrated by enzymes (e.g., the RuvAB complex) along homologous DNA sequences and must be restored into linear duplexes by HJRs (e.g., RuvC) to complete recombination (49, 59). Such recombination activities are essential both for rescue of inactivated DNA replication forks during cell division and for integration of exogenous DNA during transformation (49). Since the (HJ) helicase RuvB is a component of the RuvABC resolvosome complex (75), which resolves HJ, such resolution may be critical for transformation by exogenous DNA (30, 32).

Immediately downstream of dprA, the open reading frame (ORF) HP0334, which we (77) and others (27) now call dprB, has been annotated to encode a putative HJR (14, 71). Considering the potentially related functions in natural transformation of dprA and dprB, we examined whether the dprB product participates in transformation and recombination. Using in silico methods, Arrivd et al. identified a group of putative HJRs related to Escherichia coli YggF (7), and recently, using single-mutation construction, Humbert et al. found that H. pylori DprB is involved in natural transformation rather than in DNA repair (27). Here, by in silico, biochemical, and genetic analyses, we show that H. pylori DprB and E. coli YggF belong to the same novel protein family proposed to be functional HJRs. Our analysis with double-mutant construction confirms and extends the previous observations by showing that although there is overlap in function, DprB and RuvC preferentially affect transformation and DNA repair, respectively, in H. pylori. These findings suggest pathway specificity for the multiple HJRs in each cell.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. The H. pylori and E. coli strains and plasmids used in this study are listed in Table S1 in the supplemental material. The H. pylori strains were grown at 37°C in 5% CO₂ on Trypticase soy agar plates with 5% sheep blood (TSA; BBL Microbiology Systems, Cockeysville, MD) or Brucella agar plates (BA; Difco Laboratories, Detroit, MI) supplemented with 10% newborn calf serum.
(NBCS; Serologicals Corporation, Norcross, GA) (40). Antibiotic-resistant H. pylori mutant strains were selected with kanamycin (Km) (10 \( \mu \)g/ml), chloramphenicol (Cm) (10 \( \mu \)g/ml), streptomycin (Str) (25 \( \mu \)g/ml), or rifampin (Rif) (7.5 \( \mu \)g/ml) as appropriate. E. coli strains were grown in Luria-Bertani (LB) medium at 37°C (54). Ampicillin (Ap) (100 \( \mu \)g/ml), Km (50 \( \mu \)g/ml), or Cm (30 \( \mu \)g/ml) was used for selecting vectors or constructed plasmids in E. coli during cloning.

In silico analysis of DprB and RuvC homologs. Amino acid sequences of HP0334 (DprB), HP0877 (RuvC), and their homologs were retrieved from GenBank (www.ncbi.nlm.nih.gov). Homology analysis was performed with PSI-BLAST (3). Amino acid sequences were aligned using Vector NTI (Invitrogen, Carlsbad, CA), and phylogenetic trees were generated using Clustal X 2.0 (38) and then visualized with TreeView (52). Divergence of amino acid sequences based on pairwise distances was calculated with DIVEIN (19). Comparison of expected protein structures of DprB and RuvC was generated using the SWISS-MODEL automatic modeling mode (9).

Reverse transcription-PCR. H. pylori cell pellets were prepared from cultures grown for 48 h on TSA plates. Total RNA was isolated with the RiboPure bacteria kit (Ambion, Inc., Austin, TX), and DNA was further removed with RNase-free DNase I (Qiagen, Valencia, CA). Reverse transcription was performed with 1 \( \mu \)g of DNase-treated total RNA using the Retroscript kit (Ambion) according to the manufacturer’s instructions with pairs of specific primers (see Fig. 2; also see Table S2 in the supplemental material).

Construction of H. pylori mutants of dprA, dprB, and dprAB. Mutations of dprA (HP0333), dprB (HP0334), and dprAB (HP0333 and HP0334) were constructed by replacing the ORFs with a cat cassette (without out disrupting the ORF of the upstream or downstream gene) through homologous recombination, as described below (see Fig. S1 in the supplemental material). A 1,005-bp fragment upstream of dprB (dprBup) was obtained by PCR using genomic DNA from the H. pylori wild-type strain 26695 as the template and primers 0334L-F-SacII and 0334L-R-SpeI (see Table S2 in the supplemental material), which insert SacII and SpeI restriction sites at the ends of the fragment, respectively. Similarly, an 806-bp fragment downstream of dprB (dprBdown) was obtained by PCR using primers 0334R-F-Spel and 0334R-R-PstI (see Table S2), which insert the Spel or PstI restriction sites at the ends of the fragment. Fragments dprBup and dprBdown were digested with SacII/SpeI and Spel/PstI, respectively, and then ligated into SacII/SpeI-digested pGEM-T Easy (Promega, Madison, WI), creating pXZ056 (see Table S1). Similarly, a 1,115-bp fragment upstream of dprA (dprAup) was obtained by PCR using primers 0333L-F-SacII and 0333L-R-SpeI (see Table S2), and a 910-bp fragment downstream of dprA (dprAdown) was obtained by PCR using primers 0333R-F-Spel and 0333R-R-PstI (see Table S2). Fragments dprAup and dprAdown were digested with SacII/SpeI and Spel/PstI, respectively, and then ligated into SacII/SpeI-digested pGEM-T Easy, creating pXZ058 (see Table S1). Fragments dprAup and dprBdown were digested with SacII/SpeI and Spel/PstI, respectively, and then ligated into SacII/SpeI-digested pGEM-T Easy, creating pXZ057 (see Table S1).

The cat cassette, conferring chloramphenicol resistance (Cm’), was obtained by PCR using pAD1-Cat as the template and primers Cat-F-SpeI and Cat-R-NoT-SpeI (see Table S2 in the supplemental material). A 1,115-bp fragment downstream of dprA was obtained by PCR using pAD1-Cat as the template and primers Cat-F-SpeI and Cat-R-SpeI (or Cat-R-NoT-SpeI) (see Table S2 in the supplemental material), which insert SacII and SpeI restriction sites at the ends of the fragment, respectively (see Table S1). The cat cassette was digested with Spel and then ligated with Spel-digested plasmids pXZ056, pXZ052, and pXZ057, creating pXZ125, pXZ124, and pXZ123, respectively (see Table S1). H. pylori strain 26695 was transformed to Cm’ with pXZ123, pXZ124, and pXZ125, respectively, with pMutS2Km as the template and primers AphA-F-Spel and AphA-R-Spel (see Table S2 in the supplemental material). The aphA cassette, conferring kanamycin resistance (Km’), was obtained by PCR using pMutS2Km as the template and primers AphA-F-Spel and AphA-R-Spel, respectively, and placed downstream of the ureAB promoter on vector plasmid pAD1-Cat by the same methods as those described previously (4), leading to the creation of pXZ131, pXZ132, and pXZ133, respectively (see Table S1).

The wild-type H. pylori strain 26695 was transformed to Cm’ with pXZ124 to create mutant HPXZ337, and HPXZ342 (dprABCcat), respectively (see Table S1 in the supplemental material). Genomic DNA of these mutants was isolated and PCR performed to confirm that HPXZ337, HPXZ340, and HPXZ342 each carry the correct mutation of dprA, dprB, and dprAB, respectively, with cat cassette-specific primer Catup and genome locus-specific primer HP0333L-F (see Table S2).

Genetic complementation of dprA, dprB, and dprAB deletion mutants. Since the dprA, dprB, and dprAB deletion mutants each have decreased transformation ability, the plasmids for complementation could not be easily transformed into these mutants. To enable genetic complementation of the dprA, dprB, and dprAB deletion mutants, a two-step procedure was employed. First, we constructed strains that had an additional genomic copy of the dprA, dprB, or dprAB ORF in the urea locus. In step two, the dprA, dprB, or dprAB ORF at the native locus of each of the constructed strains was replaced by an aphA cassette through homologous recombination.

ORFs of dprA, dprB, and dprAB were first obtained by PCR using the 26695 genome as the template and primers 0333ORF-F-XbaI/0333ORF-R-Smal, 0334ORF-F-XbaI/0334ORF-R-Smal, and 0333ORF-F-XbaI/0334ORF-R-Smal (see Table S2 in the supplemental material), respectively, and placed downstream of the ureAB promoter on vector plasmid pAD1-Cat by the same methods as those described previously (4), leading to the creation of pXZ131, pXZ132, and pXZ133, respectively (see Table S1). H. pylori strain 26695 was transformed to Cm’ with pXZ123, pXZ124, and pXZ125, respectively (see Table S1). H. pylori strains HPXZ376, HPXZ377, and HPXZ378 were transformed to Km’ with plasmids pXZ123, pXZ124, and pXZ125, respectively, with pMutS2Km as the template and primers AphA-F-Spel and AphA-R-Spel, respectively, and also carry a copy of the wild-type allele (dprA, dprB, and dprAB, respectively) downstream of the ureAB promoter at the ureAB locus. As a control, HPXZ379 was constructed by transforming 26695 to Cm’ with pAD1-Cat with no H. pylori ORF insertion (see Table S1).

The aphA cassette, conferring kanamycin resistance (Km’), was obtained by PCR using pMutS2Km as the template and primers AphA-F-Spel and AphA-R-Spel, respectively, and placed downstream of the ureAB promoter on vector plasmid pAD1-Cat by the same methods as those described previously (4), leading to the creation of pXZ131, pXZ132, and pXZ133, respectively (see Table S1).

Construction of the ruvC mutant and the dprB ruvC double mutant. Similar to the other mutant constructions, fragments upstream (ruvCup) of ruvC (HP0877) and downstream (ruvCdown) were obtained by PCR using the 26695 genomic DNA as the template and primers 0877L-F-SacII/0877L-R-Spel and 0877R-F-Spel/0877R-R-PstI, respectively (see Table S2 in the supplemental material). PCR products ruvCup and ruvCdown were digested with SacII/SpeI and Spel/PstI, respectively, and then ligated into the SacII/SpeI-digested vector pGEM-T Easy, creating pXZ148 (see Table S1). The Spel-digested cat cassette was ligated into Spel-digested pXZ148, creating pXZ150 (see Table S1). H. pylori strain 26695 was transformed to Cm’ with pXZ150 to create the ruvC::cat mutant strain HPXZ359 (see Table S1). This strain then was transformed to Km’ with pXZ124 to create the ruvC::cat dprB::aphA double mutant strain HPXZ475 (see Table S1). Genomic DNA from each mutant was isolated, and PCR was performed to confirm the correct mutant constructions.

Construction of donor DNA for homologous and homeologous transformation. An 801-bp DNA sequence encompassing H. pylori rpsL from Str’ H. pylori strain 26695 (39, 40) was obtained by PCR using primers rpsL801-F and rpsL801-R (see Table S2 in the supplemental material) and cloned into the vector pGEM-T Easy (Promega, Madison, WI) to create plasmid p801R (see Table S1), which was used as donor DNA for homologous transformation. Similarly, a 772-bp DNA sequence encompassing Helicobacter ceterum rpsL from Str’ H. ceterum strain 04-116 (see Table S1) was obtained by PCR with the same pair of primers and cloned.
into the same vector to create plasmid pB01RHC (see Table S1), which was also used as donor DNA for homologous transformation.

Construction of \( \text{dprB}::0, \text{dprB}::50, \) and \( \text{dprB}::100 \) mutants and \( \text{ruvC}::0, \text{ruvC}::50, \) and \( \text{ruvC}::100 \) mutants and assay of intragenomic recombination rate. To assess rates of intragenomic recombination of \( H. pylori \) wild-type and \( \text{dprB} \) and \( \text{ruvC} \) mutant strains, the reported deletion cassettes with 0, 50, or 100 bp of identical DNA sequences (IDS) (6) were obtained by PCR using plasmids pUREA0, pUREA50, and pUREA100 (see Table S1 in the supplemental material) as the template, respectively, and PCR was performed using the primer pair ApHA-F-SpeI and ApHA-R-SpeI (see Table S2). The PCR products of the cassettes with flanking 0-, 50-, and 100-bp repeats were digested with SpeI and then ligated into SpeI-digested pXZ052, creating pXZ113, pXZ114, and pXZ115, respectively (see Table S1). \( H. pylori \) strain 26695 was transformed to \( \text{Cm}^\text{r} \) with pXZ113, pXZ114, and pXZ115 to create strains HPXZ331 (\( \text{dprB}::0 \)), HPXZ334 (\( \text{dprB}::50 \)), and HPXZ337 (\( \text{dprB}::100 \)), respectively (see Table S1). HPXZ337 is the \( \text{dprB} \) mutant with a deletion cassette with 100-bp repeats replacing the \( \text{dprB} \) ORF, HPXZ334 is a \( \text{dprB} \) mutant with a 50-bp repeat deletion cassette, and HPXZ331 is a \( \text{dprB} \) mutant with no repeats flanking the cassette. A set of \( \text{ruvC} \) mutants with deletion cassettes (strains HPXZ2764 (\( \text{ruvC}::0 \)), HPXZ2767 (\( \text{ruvC}::50 \)), and HPXZ2770 (\( \text{ruvC}::100 \)) were constructed similarly; these are the \( H. pylori \) 26695 \( \text{ruvC} \) mutants with 0-, 50-, and 100-bp repeats, respectively, in the deletion cassette replacing the \( \text{ruvC} \) ORF (see Table S1). Control strains HPXZ540 (\( \text{vacA}::0 \)), HPXZ543 (\( \text{vacA}::50 \)), and HPXZ554 (\( \text{vacA}::100 \)) were constructed similarly, and they are \( H. pylori \) 26695 with 0-, 50-, and 100-bp repeats, respectively, in the deletion cassette replacing the \( \text{vacA} \) ORF (see Table S1). To assess deletion frequencies, the constructed strains were grown on TSA plates for 48 h at 37°C in 5% CO₂, allowing for deletions to occur, and then cells were collected in Brucella broth (BB) medium and washed twice with BB medium, and serial dilutions of cell suspensions in 200 µl of BB medium were spread on BA plates containing 10% NBCS and 25 µg/ml streptomycin. The plates were incubated for 4 days at 37°C in 5% CO₂, and the transformation frequency was determined by the number of surviving colonies on the plates, which were achieved by using BBL CampyPak Plus microaerophilic plates (Becton, Dickinson, and Company, Sparks, MD). For each transformation treatment, \( H. pylori \) strains with no DNA added also were examined in parallel as a negative control; no colonies were seen in any case. Each experiment was repeated three to six times with independent cultures.

Genetic complementation of \( \text{E. coli} \) \( \text{ruvC} \) mutants. To evaluate the functions of the \( H. pylori \) \( \text{dprB} \) (HP334) and \( \text{ruvC} \) (HP0877) gene products, a set of plasmids was constructed to express \( H. pylori \) \( \text{Ruvc} \) or \( \text{DprB} \) in an \( E. coli \) \( \text{ruvC} \) mutant background. Linear DNA PCR products containing the C-terminal \( \text{His}_6 - \)tagged target gene ORF with an upstream T7 promoter and ribosomal binding site and a downstream T7 terminator were generated with the EasyXpress linear template kit (Qiagen) and then ligated into vector pGEM-T Easy (Promega) in the same direction to give plasmids p801R or p801RHc (50 ng) (see Table S1 in the supplemental material). Each transformation mixture with recipient cells (100 µl) and donor DNA pB01RHC or pB01RH (50 ng) (see Table S1) was grown in LB liquid medium with Ap at 37°C to an OD_{600} of 1.0. Cell cultures (1 ml) were collected, and after suitable dilutions in PBS, equal suspensions were inoculated onto LB plates. When dried, plates were exposed to 254-nm UV generated by a USHIO G30T UV lamp (Fisher Scientific, Gaithersburg, MD) at a distance of 78 cm for 0 to 60 s (resulting in a dose rate of about 2.0 J/m²/s) and then were incubated at 37°C in 5% CO₂ for 4 days before colonies were counted and survival rates determined. Each experiment was repeated three times with independent cultures. To evaluate \( E. coli \) strain recovery from DNA damage by UV irradiation, each \( E. coli \) strain was grown in LB liquid medium with Ap at 37°C to an OD_{600} of 1.0. Cell cultures (1 ml) were collected, and after suitable dilutions in PBS, equal suspensions were inoculated onto LB plates with Ap. Plates were exposed to 254-nm UV (USHIO G30T UV lamp; Fisher Scientific) at a distance of 78 cm for 0, 20, or 40 s and then incubated at 37°C for 24 h before survival rates were determined.

Susceptibility to ciprofloxacin and levofloxacin was evaluated using ciprofloxacin and levofloxacin Etest strips (bioMérieux, Hazelwood, MO), respectively, according to the manufacturer’s instructions. Briefly, cells from each \( H. pylori \) culture on a TSA plate with 48 h of growth were transferred to a new TSA plate, and Etest strips were applied. Plates were incubated for 48 h at 37°C in 5% CO₂, and MICs were determined in at least six independent experiments.

Susceptibility to ROS was evaluated as described previously (33), with methyl viologen (MV) (Sigma-Aldrich, St. Louis, MO) used as an ROS source. Briefly, cells from each 48-h \( H. pylori \) culture on a TSA plate were resuspended to an OD_{600} of 0.5 in BB liquid medium containing 10% NBCS with either MV (10 nM) or PBS (a control). Liquid cultures were grown with shaking (90 rpm) for 24 h at 37°C in microaerophilic conditions, which were achieved by using BBL CampyPak Plus microaerophilic system envelopes (BBL Microbiology Systems, Becton, Dickinson, MD). They were then serially diluted and incubated onto TSA plates for 4 days at 37°C in 5% CO₂, and survival rates were calculated based on three independent experiments.

Holliday junction structure synthesis and protein synthesis. Model Holliday junction HJ-X4 (X4) was formed by annealing four 50-mer oligonucleotides, HJ-X4-S1, HJ-X4-S2, HJ-X4-S3, and HJ-X4-S4 (see Fig. S7B and Table S2 in the supplemental material), as described previously (72). Another model Holliday junction (H1-HpX12) was formed in the same way with four 50-mer oligonucleotides: strands Hpx12-S1, Hpx12-S2, Hpx12-S3, and Hpx12-S4 (see Fig. S7B and Table S2). Strand
HPX12-S1 is a sequence of H. pylori strain 26695 with genome location 1,268,032 to 1,268,081. HJ-X4-S1 and HJ-HpX12-S1 strands were 5′-biotin-labeled or 5′-6-carboxyfluorescein (FAM)-labeled oligonucleotides supplied by Sigma-Aldrich Co (St. Louis, MO). A 50-bp segment of duplex (double-stranded) DNA was formed by annealing two oligonucleotides, HJ-X4-S1 and HJ-X4-S1-reverse (see Table S2). The 5′-labeled HpX12-S1 was used as a single-stranded DNA substrate. C-terminal His-tagged H. pylori DprB protein, E. coli RuvC protein, and H. pylori RuvC protein were synthesized using a EasyXpress linear template kit (Qiagen) and EasyXpress protein synthesis minikit (Qiagen), and results were confirmed by Western bloting using mouse monoclonal anti-polyhistidine antibody (Sigma) as the primary antibody and goat anti-Mouse IgG-alkaline phosphatase antibody (Sigma) as the secondary antibody. The target protein concentration in this in vitro synthesis system was estimated by comparing total protein concentrations before and after synthesis using the Bradford protein assay (64).

Holliday junction DNA binding assay. To determine whether the dprB gene product binds Holliday junction structures in vitro, biotin-labeled synthesized DNA substrates (50 fmol) or FAM-labeled synthesized DNA substrates (500 fmol) were mixed on ice for 15 min with the in vitro-synthesized DprB protein (0.5 μg) in 20 μl binding buffer (50 mM Tris-HCl, pH 8.0, 5 mM EDTA, 1 mM dithiothreitol [DTT], 100 μg/ml BSA) (57). The binding reaction mixtures were loaded on a 6% DNA retardation gel (Invitrogen), and electrophoresis was performed using 0.5X TBE (0.045 M Tris-borate, 0.001 M EDTA) buffer at 100 V for 1 to 1.5 h at 4°C. The biotin-labeled samples were transferred to a nylon membrane (Roche Diagnostics GmbH, Mannheim, Germany) using a Trans-Blot electrophoretic transfer cell (Bio-Rad Laboratories, Hercules, CA), and 3′-biotin-labeled DNA was detected with the LightShift chemiluminescent electrophoretic mobility shift assay (EMSA) kit (Pierce, Rockford, IL). The FAM-labeled samples were detected by fluorescence using a GE Typhoon scanner (GE Healthcare Life Sciences, Fairfield, CT).

Results

A new protein family in Gram-negative bacteria. In H. pylori strain 26695, ORF HP0334 has been annotated to encode a 134-amino acid putative protein resembling the known Holliday junction resolvase (HJR) RuvC (71) with 15% identity. BLAST analysis indicates that H. pylori HP0334 has homologs in many other Gram-negative bacteria, with identities from 25 to >90%, although none has a known function. No homolog in any Gram-positive bacterial species was identified. H. pylori HP0334 shares 29% identity with an E. coli protein (YggF) encoded by yggF (b2949) (11) that also has been annotated as an HJR-like protein and was found to be essential for growth in complex media (11, 24). E. coli YggF is similar to but distinct from RuvC in secondary structure (7, 41). Amino acid sequence alignment of HP0334 from H. pylori 26695 and its homologs from other sequenced H. pylori strains the closely related H. hepaticus and Campylobacter jejuni, as well as Haemophilus influenzae, E. coli, and Legionella pneumophila, indicates that all possess shared N-terminal domains, suggesting conserved function (see Fig. S3 in the supplemental material). Alignment of HP0334 homologs and RuvC homologs from 15 bacterial species possessing both genes indicates similarity of the N-terminal conserved domains between the two protein families (see Fig. S4), but this conserved N-terminal domain is not present in archaeal HJR (data not shown). The RuvC and HP0334 homolog families are clearly distinct, as is another E. coli HJR (RusA) (Fig. 1), suggesting divergence from a common ancestor (7). The mean (± standard deviations) pairwise diversity between the 15 bacterial RuvC homologs (1.38 ± 0.42) was consistently less than that for the HP0334 homologs in the same strains (1.73 ± 0.43; P < 10−22 by paired t test), consistent with greater functional constraint than for HP0334 or more recent divergence from the ancestral HP0334-like protein. In total, these analyses provide evidence for a new protein family with possible HJR function in Gram-negative bacteria.

Structural models of HP0334 and RuvC. Using the E. coli hypothetical protein YggF (41) and the E. coli RuvC protein (8) as the templates, analyses of H. pylori HP0334 and the presumed H. pylori HJR, RuvC (HP0877), were performed using the SWISS-MODEL program (see Fig. S5 in the supplemental material). As expected, the predicted secondary structures of H. pylori and E. coli RuvC are highly similar (8); both are composed of 5 β-sheets and 5 α-helices arranged in the same pattern (see Fig. S3). The predicted secondary structures of H. pylori HP0334 and E. coli YggF (41) also are highly similar; both are composed of 5 β-sheets and 3 α-helices arranged in the same pattern. For H. pylori HP0334, the predictions are β1(1-5)-β2(13-18)-β3(21-25)-α1(38-45)-β4(51-55)-α2(64-73)-β5(80-82)-loop-α3(118-129), with virtual tertiary structure identity with E. coli YggF (see Fig. S5), further suggesting functional similarity (41). Compared to H. pylori HP0334 and E. coli YggF, the RuvC proteins of H. pylori and E. coli have two additional α-helices between the β-sheet and C-terminal α-helix.

Genomic localization of H. pylori HP0334. In H. pylori, HP0334 is located downstream of dprA (HP0333), overlapping by four bases, and is transcribed in the same orientation (Fig. 2). This same general organization is observed in all sequenced H. pylori strains and in the closely related H. hepaticus, H. acnonychus, C. jejuni, Campylobacter fetus, and Wolinella succinogenes (see Fig. S6 in the supplemental material). Most of these species (except W. succinogenes and C. fetus) are known to be naturally competent for DNA uptake (53, 67, 74). Reverse transcription-PCR (RT-PCR) confirmed that dprA and HP0334 are cotranscribed as a bicistronic operon, since the upstream and downstream genes are not also cotranscribed (Fig. 2). Such observations suggest that HP0334 is functionally related to DprA in H. pylori and in the other closely related and naturally competent bacterial species.

Role of dprB in H. pylori natural competence. Interruption of dprA leads to a 2-log10-fold decrease in H. pylori transformation (4). To determine whether dprB also plays a role in transformation, plasmid p801R, carrying an 801-bp H. pylori 26695 rpsL fragment containing a point mutation (A128G) that converts an H. pylori Str+ strain to Str− (39), was used as a donor to transform the wild-type and mutant H. pylori strains. In our standard test conditions (39), the transformation frequency (TF) of the wild-type strain 26695 was ~10−3. Deletion of dprA decreased the H. pylori TF by 2.1 log10-fold (P < 0.05) (Fig. 3A), consistent with our prior finding (4); dprB deletion significantly decreased the H. pylori TF by 0.5 log10-fold (P < 0.05) (Fig. 3A). Deletion of both dprA and
dprB decreased the TF by 2.5 log_{10}-fold ($P < 0.05$) (Fig. 3A). In complementation experiments, the TF of the control strain HPXZ397 (26695 *ureAB*-*cat*) was $6.5 \times 10^{-4} \pm 3.5 \times 10^{-4}$, similar to that of the parental wild-type 26695, indicating that the interruption of the *ureAB* locus for complementation and expression of the *cat* cassette does not affect natural transformation frequencies. Expressing dprB in trans under the *ureAB* promoter in the dprB mutant restored the TF to wild-type levels of transforma-

![Neighbor-joining phylogeny generated from alignment of the products of dprB (HP0334) and ruvC (HP0877) in 15 bacterial species. The 15 species selected have an ortholog of each gene. E. coli Holliday junction resolvase RusA was used as an outgroup to root the tree. For the HP0334 family, the asterisk indicates that the HP0334 ORF or its homolog has a genomic position adjacent to dprA or its homolog. Because HP0334 and its orthologs clearly represent a conserved gene family in Gram-negative bacteria, we designate them the dprB family based on their close relationship with dprA in some strains.](http://jb.asm.org/)

![Analysis of dprB transcription. Analyses were performed by RT-PCR of cDNA and by PCR using genomic DNA as a positive control and RNA without reverse transcription (RT) as a negative control. In PCR-1 (primer pair 1/4F and 1/2/3R), the expected 0.4-kb product was obtained; in PCR-2 (primer pair 2F and 1/2/3R), a 1.2-kb product was obtained; in PCR-3 (primer pair 3F and 1/2/3R), no product was obtained; and in PCR-4 (primer pair 1/4F and 4R), no product was obtained.](http://jb.asm.org/)
Did not significantly decrease the ruvC terminator-less dprA mutation was constructed by replacing the ORF with a deletion cassette that has no (0-bp) IDS, no intragenomic recombination. Similarly, the deletion frequencies of the ruvC mutants with the 50- and 100-bp IDS were significantly lower than that in the 0-bp IDS, no deletions were detected under the assay conditions (*, relative frequency of <10⁻⁴).

The explanation for these observations is that the dprA mutation has dramatically (3.7 log₁₀-fold [P < 0.05]) decreased the homologous TF (Fig. 3B), which is similar to its decreasing of the homologous TF (0.5 log₁₀-fold). No homeologous transformation event was observed for the dprAB double mutant (indicating a >4.0 log₁₀-fold decrease) in the standard assay. Thus, dprAB operon function is required for homeologous transformation.

Deletion of ruvC did not significantly decrease the homeologous TF (Fig. 3B), but no homeologous transformation event was observed for the ruvC dprB double mutant. These data differentiate between dprB and ruvC but suggest the requirement for HJR function in H. pylori homeologous transformation.

Deletion of dprB decreases H. pylori intragenomic recombination. To determine whether DprB plays a role in the efficient use of repetitive DNA by H. pylori in intragenomic recombination (6), we examined the effect of dprB mutation on the rate of deletion by using engineered genomic cassettes with identical DNA sequences (IDS) of 0, 50, or 100 bp (6) replaced vacA (control), ruvC, or dprB in the H. pylori 26695 background. For the vacA, ruvC, and dprB mutants with no (0-bp) IDS, no deletions were detected under the assay conditions (*, relative frequency of <10⁻⁴).

![FIG 3](image-url) Transformation frequencies of H. pylori mutants evaluated by deletion frequency assay. A chloramphenicol resistance cassette flanked by identical DNA sequences (IDS) of 0, 50, or 100 bp (6) replaced vacA (control), ruvC, or dprB in the H. pylori 26695 background. For the vacA, ruvC, and dprB mutants with no (0-bp) IDS, no deletions were detected under the assay conditions (*, relative frequency of <10⁻⁴).
DprB Facilitates Inter- and Intragenomic Recombination

log_{10}-fold (P < 0.05) and 0.4 log_{10}-fold (P < 0.05), respectively (Fig. 4). Thus, RuvC and DprB functionally overlap in H. pylori intragenomic recombination with a stronger effect for dprB.

DprB facilitates H. pylori DNA damage repair. Since homologous recombination is important in the repair of DNA damage, functional HJRs may play critical roles by resolving the HJ structures formed during the process (76). To determine whether DprB contributes to DNA repair, we examined the susceptibility to UV irradiation of H. pylori wild-type and ΔdprB strains. In a control experiment, an H. pylori JP26 ruvB mutant showed significantly decreased survival compared to that of the wild type (Fig. 5A), consistent with prior reports (32). Under the same conditions, dprB deletion significantly (1 log_{10}-fold; P < 0.05) decreased H. pylori 26695 survival compared to the wild type (Fig. 5B). Deletion of dprA did not significantly affect H. pylori 26695 survival, while deletion of both dprA and dprB decreased 26695 survival to rates similar to that of deletion of dprB alone (Fig. 5B). In complementation studies, expressing dprB in trans under the ureAB promoter (PureAB) in the dprB mutant restored survival to the wild-type level, and expressing dprAB under the same promoter in the dprAB double mutant also restored survival (Fig. 5C). These results indicate that DprB participates in DNA damage repair without the involvement of the upstream cotranscribed dprA and to a lesser extent than by RuvB (Fig. 5A and B), suggesting that H. pylori RuvC also participates in DNA repair. To test this hypothesis, we constructed ruvC and dprB ruvC mutants. Mutation of ruvC increased susceptibility to UV exposure to a greater extent than for dprB (Fig. 5D). The double mutant had dramatically diminished UV survival, significantly lower than that of the single dprB or ruvC deletion mutants at all levels of exposure (Fig. 5D).

The fluoroquinolones ciprofloxacin and levofloxacin target bacterial gyrase and type II and type IV topoisomerases, both inhibiting DNA synthesis and inducing DNA damage (13). Confirming a prior report (45), the deletion of ruvC significantly decreased the MICs of both ciprofloxacin and levofloxacin (Table 1), indicating its role in the recombinational repair of fluoroquinolone-induced DNA damage. Deletion of dprB in strain 29965 also significantly decreased the MICs of both ciprofloxacin and levofloxacin (Table 1); in trans complementation restored susceptibility (Table 1), indicating a dprB role in recombinational repair. Deletion of dprAB significantly decreased the MICs of both agents to an extent similar to that for dprB alone, indicating a minimal role of dprA in this repair. dprB ruvC double deletion dramatically decreased the MIC of both ciprofloxacin and levofloxacin compared to those against the single dprB or ruvC deletion mutant (Table 1). In total, these results indicate that DprB functionally overlaps with RuvC, which plays known roles in DNA recombinational repair (47). However, after exposure to reactive oxygen species (ROS), as described previously (33), there was no difference in survival rate among the wild-type strain (−1.7 log_{10}) or

![Figure 5](https://example.com/figure5.png)

**FIG 5** Survival of H. pylori mutants after UV exposure. H. pylori cells on TSA plates were subjected to a range of UV exposures, and surviving proportions were determined. (A) H. pylori JP26 ruvB mutant versus wild-type JP26. (B) H. pylori wild-type 26695 and ΔdprA, ΔdprB, and ΔdprAB mutants. (C) The complemented strains of ΔdprA, ΔdprB, and ΔdprAB mutants and the wild-type control strain [26695(pAD1-Cat)]. (D) H. pylori wild-type 26695 and ΔdprB, ΔruvC, and ΔdprB ΔruvC mutants.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>MIC_{c} (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>26695</td>
<td>Wild type</td>
<td>0.057 ± 0.009</td>
</tr>
<tr>
<td>HPXZ337</td>
<td>ΔdprA</td>
<td>0.051 ± 0.011</td>
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<tr>
<td>HPXZ340</td>
<td>ΔdprB</td>
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<tr>
<td>HPXZ342</td>
<td>ΔdprAB</td>
<td>0.038 ± 0.008*</td>
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<tr>
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<td>ΔruvC</td>
<td>0.042 ± 0.007</td>
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<tr>
<td>HPXZ475</td>
<td>ΔdprB ΔruvC</td>
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<tr>
<td>HPXZ418</td>
<td>ΔdprB + dprAB^c</td>
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</tr>
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* Each experiment was repeated ≥5 times.

^a The plus sign indicates in trans complementation.

^a, b, c, P < 0.05 compared to the wild type.
ing that DprB and RuvC play different roles in recombinational repair, an event that may frequently occur during chromosomal DNA replication (49).

**Overexpression of *E. coli* and *H. pylori* HJRs in *E. coli*.** For cross-species complementation in *E. coli*, *H. pylori dprB* (HP0334) and *ruvC* (HP0877), and *E. coli yqgF* (b2949) and *ruvC* (b1863), each was individually fused with a T7 promoter and ribosome binding site (RBS) and then cloned into the multiple-copy vector pGEM-T Easy in the same orientation (see Fig. S2A in the supplemental material). The T7 RNA polymerase-bearing *E. coli* strains BL21(DE3) and BL21(DE3)pLysS (65) could not be transformed by the multiple-copy vector with the cloned *H. pylori ruvC*, *E. coli ruvC*, or *E. coli yqgF*, even without isopropyl-β-D-thiogalactopyranoside (IPTG) induction, but were transformed by the same vector with the cloned *H. pylori dprB* (see Fig. S2D in the supplemental material). However, the *E. coli* wild-type BW25113 strain, lacking T7 RNA polymerase (11), could be transformed by the multiple-copy vector expressing *H. pylori RuvC*, *E. coli RuvC*, *E. coli YqgF*, or *H. pylori DprB* (see Fig. S2D). These results indicate that overexpression of *H. pylori RuvC*, *E. coli RuvC*, or *E. coli YqgF* is toxic in *E. coli*, consistent with the prior observation of the toxicity of overexpressing *E. coli RuvC* and *YqgF* (37).

**H. pylori RuvC complements *E. coli ruvC* mutation.** Our results showed that the predicted *H. pylori* HJR RuvC (HP0877) has DNA repair functions, and protein predictions (see Fig. S5 in the supplemental material) indicate that it has a predicted three-dimensional (3D) structure similar to that of the *E. coli* RuvC protein (8). To confirm *H. pylori RuvC* function, cross-species complementation was performed by transforming an *E. coli* BW25113 *ruvC* mutant (11), which has increased UV sensitivity (47), with a multiple-copy plasmid expressing *H. pylori ruvC* (Fig. 7A). Complementation with *H. pylori ruvC* restored survival to the wild-type level, similar to that restored by *E. coli ruvC* expressed in the same plasmid (Fig. 7A). These results confirm that the putative *H. pylori RuvC* has a function similar to that of *E. coli RuvC*. *H. pylori dprB* expressed from the same plasmid provided partial restoration of UV resistance (Fig. 7A), indicating similarities to and differences from RuvC function.

**Nonreciprocal *E. coli yqgF* and *H. pylori dprB* complementation.** The *H. pylori* HJR-like DprB and its homolog in *E. coli* (*YqgF*) have nearly identical predicted 3D protein structures (see Fig. S5 in the supplemental material). However, *yqgF* is essential in *E. coli* (24), while *dprB* is not essential in *H. pylori*. To further investigate *H. pylori DprB* and *E. coli yqgF* functions, cross-species complementation was performed by expressing *E. coli yqgF* in trans under the *ureAB* promoter in the *H. pylori dprB* mutant. *E. coli YqgF* partially restored survival of the UV-exposed *H. pylori dprB* mutant (Fig. 7B), indicating functional complementation. We next examined whether *H. pylori DprB* can replace the essential *YqgF* function in *E. coli*. Supplying additional copies of *E. coli yqgF* in a shuttle plasmid allowed us to interrupt the essential genomic *yqgF* in the *E. coli* wild-type strain BW25113 (see Fig. S2B and C), which had not been achieved before (11). However, supplying additional copies of *H. pylori dprB* in trans in the same plasmid background failed to permit interruption of the essential *E. coli yqgF* (see Fig. S2B). This also is consistent with our observation (see Fig. S2D) that overexpressing *E. coli yqgF*, but not *H. pylori dprB*, is toxic to *E. coli*. Thus, the requirements for *yqgF* function in *E. coli* are more specific than those for *DprB* in *H. pylori*.
DprB specifically binds Holliday junctions. The E. coli HJR RuvC specifically binds HJ structures \((21, 56, 68, 72)\). To investigate whether DprB is a functional HJR, we examined whether it binds in vitro to synthesized HJ structures. C-terminal His-tagged E. coli RuvC, H. pylori RuvC, and H. pylori DprB were synthesized in vitro; all three proteins have similar molecular masses \((15 \text{ to } 20 \text{ kDa})\) (see Fig. S7A in the supplemental material). Electrophoretic mobility shift assay (EMSA) indicated that E. coli RuvC, H. pylori RuvC (data not shown), and H. pylori DprB did not bind single-stranded DNA, confirming prior studies \((68)\) (Fig. 8B and E). The H. pylori RuvC protein also specifically bound to the synthetic HJ structure HJ-X4 but did not bind duplex DNA, confirming prior studies \((68)\) (Fig. 8B and E). The H. pylori RuvC protein specifically bound to the HJ structure HJ-X4 but did not bind duplex DNA, confirming prior studies \((68)\) (Fig. 8B and E). The H. pylori RuvC protein also specifically bound to HJ-X4 (Fig. 8C) and did not bind duplex DNA (data not shown). As with the RuvC HJR proteins, DprB did not bind duplex DNA but specifically bound to HJ-X4 (Fig. 8D and F). That the binding could be competed with unlabeled HJ-X4 rather than unlabeled nonspecific duplex competing DNA confirmed that the binding is HJ structure specific.

**DprB may be a functional Holliday junction resolvase.** To investigate whether DprB is a functional HJR, we examined whether it binds in vitro to synthesized HJ structures. C-terminal His-tagged E. coli RuvC, H. pylori RuvC, and H. pylori DprB were synthesized in vitro; all three proteins have similar molecular masses \((15 \text{ to } 20 \text{ kDa})\) (see Fig. S7A in the supplemental material). Electrophoretic mobility shift assay (EMSA) indicated that E. coli RuvC, H. pylori RuvC (data not shown), and H. pylori DprB did not bind single-stranded DNA, confirming prior studies \((68)\) (Fig. 8B and E). The H. pylori RuvC protein also specifically bound to the synthetic HJ structure HJ-X4 but did not bind duplex DNA, confirming prior studies \((68)\) (Fig. 8B and E). The H. pylori RuvC protein specifically bound to the HJ structure HJ-X4 but did not bind duplex DNA, confirming prior studies \((68)\) (Fig. 8B and E). The H. pylori RuvC protein also specifically bound to HJ-X4 (Fig. 8C) and did not bind duplex DNA (data not shown). As with the RuvC HJR proteins, DprB did not bind duplex DNA but specifically bound to HJ-X4 (Fig. 8D and F). That the binding could be competed with unlabeled HJ-X4 rather than unlabeled nonspecific duplex competing DNA confirmed that the binding is HJ structure specific.

**DISCUSSION**

The DprB Holliday junction resolvase-like family and RuvC Holliday junction resolvase family are distinct but closely related. Our in silico analysis of dprB and related genes in other species indicate that they encode a novel HJR-like family (DprB family), corresponding to the previously identified Yqgf homologs \((7)\). The DprB family may have a common ancestor with the RuvC family (Fig. 1), since they share conserved N-terminal domains (see Fig. S4 in the supplemental material) that may be involved in HJ cleavage \((60)\). Prior in silico analyses revealed similarity between the RuvC and Yqgf families \((7)\), and nuclear magnetic resonance (NMR) spectroscopy indicated structural similarity between E. coli RuvC and hypothetical E. coli protein Yqgf \((b2949)\) \((41)\). The greater degree of variation and simpler structure could indicate that dprB genes evolved from the common ancestor before ruvC.

The DprB and RuvC families functionally overlap but have different roles. By analyzing single and double deletion mutations, we observed that H. pylori DprB and RuvC functionally overlap with respect to exogenous DNA transformation, intragenomic recombination between direct DNA repeats, and repair from DNA damage induced by UV or fluoroquinolone exposure (Fig. 10). These findings reflect the known important roles of HJRs in bacteria \((49)\). However, why do a number of Gram-negative bacterial species possess two distinct HJRs that appear to have the same substrate specificity and efficiency, as exemplified by H. pylori RuvC and DprB? Possible explanations include that the DNA targets for their activity differ and that the stimuli for their expression differ. In H. pylori, there is evidence for both possibilities.

Our studies show that for repair of DNA damage (e.g., the pyrimidine dimer lesions caused by UV irradiation), both RuvC and DprB provide partial function, but in their mutual absence, survival is markedly impaired (Fig. 5). These studies show a more substantial role for RuvC than for DprB, consistent with its known role in rescue of stalled replication forks \((48)\). However, for the fluoroquinolone-induced DNA damage, the contributions of DprB and RuvC to recombinational repair appear equivalent (Table 1), and neither has any role in cell survival after ROS exposure, since the major target of ROS may not be DNA molecules, as is often supposed. Our results are consistent with the recent finding on the mechanism of ROS killing bacteria which suggests that ROS inhibits bacterial growth primarily by damaging extracytoplasmic targets \((16)\).

As anticipated, HJR function is critical for natural transformation (Fig. 3), but in contrast to DNA repair, DprB function predominates over RuvC, especially for homologous DNA. That dprB is more critical for transformation may reflect that DprB is more efficient in binding and resolving junctions formed by invading DNA. Consistent with different cellular roles of DprB and RuvC, NMR spectroscopy indicated that E. coli RuvC is dimeric but Yqgf, the DprB homolog, is monomeric \((41)\). Monomeric DprB and dimeric RuvC may differ in their affinity to differing DNA structures within bacterial cells. Microarray studies showed that dprB and ruvC have similar transcription levels in free-living H. pylori 26695 strains \((44)\), suggesting that differences in function between the two proteins, rather than abundance, account for the differing phenotypes.

Another possibility is that the stimuli that induce their tran-
H. pylori ruvC, but not dprB, is under Fur repression (25). In prior studies (44), with increasing (0.25 to 1.5%) salt concentrations, transcription of H. pylori dprB rose (1.3-fold; 1.4-fold for dprA), whereas ruvC transcription fell (by 1.2-fold). Transcription of ruvC, but not dprB, was >2-fold induced in H. pylori grown with eukaryotic cells (58). Thus, possessing genes with similar functions but regulated by differing stimuli may provide enhanced flexibility for H. pylori.

In a recent study, deletion of dprB in H. pylori G27-derived strain NSH57 decreased transformation frequency and reduced growth (27), consistent with our observation of deletion of dprB in H. pylori 26695. Deletion of dprB did not affect H. pylori NSH57.
recovery from UV exposure in that study (27), whereas we found effects in strain 26695 for dprB and ruvC and even stronger effects in the strain with both mutations (Fig. 5). These different observations may be due to the different experimental conditions or the different H. pylori strains used. In our test conditions, with 80 J/m² exposure to UV (254 nm), the survival rate of wild-type H. pylori 26695 was 0.01, and no survival was observed with 1,000 J/m² exposure. In contrast, in the other study, with 1,000 J/m² exposure to UV, survival of the H. pylori NSH57 wild-type strain was >0.1 (27). H. pylori represents a highly diverse bacterial species at the genomic level, with major phenotypic variation of isolates, for example, in natural competence (5), host colonization (1), and survival under host stress conditions (34). We cannot rule out the possibility that diversity in DNA repair-related loci aside from ruvC and dprB varies the roles of DprB in different H. pylori genetic backgrounds. To further reveal DprB roles, identifying DprB protein partners and further exploring the partners’ strain specificity may be necessary.

DprB family members may vary in their roles in different bacterial genera. Since we found that dprB is cotranscribed with dprA, which is needed for high-efficiency natural transformation in H. pylori (4), we examined the role of DprB in transformation. The adjacency of dprA and dprB also is observed in closely related species, some of which are naturally competent for DNA transformation (e.g., C. jejuni) and some that are not (e.g., C. fetus (53, 67, 74). However, no dprB homolog is present in another naturally transformable bacterium, Haemophilus influenzae (36), which possesses dprA only. A gene downstream of dprA in H. influenzae, which also is named dprB, is cotranscribed with dprA and its downstream dprC, but its function is unknown (35). However, H. influenzae dprB is not homologous to H. pylori HP0334 (H. pylori dprB) and has no homolog in H. pylori.

Although E. coli, which is not naturally competent, possesses a dprA homolog (smf [b4473]), its deletion does not affect transformation or DNA repair (61), and yygF (b2949) is not adjacent to smf. Even though H. pylori YygF and E. coli YygF have similar predicted protein structures (see Fig. S5 in the supplemental material), their amino acid identity is only 29%. However, that YygF appears essential in E. coli (24) and that dprB mutation only slightly reduces growth in H. pylori indicates important functional differences. Indeed, although we successfully ablated yygF from the E. coli genome with additional yygF copies in trans, we were unable to knock out yygF from the E. coli genome with additional copies of H. pylori dprB (see Fig. S2). These results indicate that H. pylori DprB and E. coli YygF are not functionally interchangeable.

Compared to E. coli, H. pylori grows slowly. The mean DNA replication speed of H. pylori (120 bp/s) is ~16-fold lower than that of E. coli (1,870 bp/s) (23). That deletion of yygF is lethal for E. coli while deletion of dprB only inhibits H. pylori growth may be related to the difference in replication speed between the two organisms. Since HJRs play crucial roles in the rescue of arrested replication forks due to damage during DNA replication (49), losing a functional HJR may be more harmful in high-speed (E. coli) than low-speed (H. pylori) DNA replication. Functional constraints due to differences in DNA replication velocity could explain why E. coli YygF can replace DprB functions in H. pylori, whereas H. pylori DprB cannot replace YygF functions in E. coli. Further study of the relationship between the DprB protein family roles and DNA replication speed may help address this hypothesis.

More interestingly, overexpression of E. coli YygF (an essential gene) or RuvC (nonessential) is toxic to E. coli, suggesting that precise regulation of these two HJRs in the rapidly growing E. coli is critical; however, in the more slowly growing H. pylori, overexpression of DprB or RuvC is not toxic, but lack of expression (with DprB expressed more than RuvC) inhibits growth. Further examination of DprB and RuvC expression can better address issues related to growth and cell division phenotypes.

FIG 10 Working model of DprB and RuvC in H. pylori. Both DprB and RuvC facilitate H. pylori homologous and homologous transformation, with DprB being more efficient in these processes. Both DprB and RuvC facilitate H. pylori intragenomic recombination between direct DNA repeats and the intragenomic recombination involved in DNA damage repair, with RuvC being more efficient in DNA damage repair. Thus, with overlapping specific activities, each enzyme has an enhanced function.
Indeed, recent examination of previously identified DNA-processing genes suggests the presence of different DNA replication mechanisms, including initiation and restarting of stalled forks, between the slow-growing 

H. pylori

and the fast-growing E. coli, although regulation of DNA replication in slow-growing bacteria is still poorly understood (51). Our identification of the novel HJR-like DprB family and the different roles of DprBs in H. pylori and E. coli provide a new direction for investigation of DNA replication and cell proliferation of slow-growing bacterial pathogens.

The greater genetic diversity we observed in DprB family members compared to RuvC family members also could reflect that DprB became species specific more rapidly; the interactions of RuvC in branch migration-resolution complexes with RuvA and DprB became species specific more rapidly; the interactions of RuvC in branch migration-resolution complexes with RuvA and RuvB may provide more substantial functional limitations (22, 72). Investigation of DprB partners in vivo, and further comparing Holliday junction resolving patterns with purified proteins and detailed mapping of cleavage sites, will assess the functional HJR role of the DprB protein. Such studies may help identify differences between RuvC and DprB, and possibly the specific roles of DprB in naturally transformable bacterial species, such as H. pylori.

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