JOURNAL OF BACTERIOLOGY, Jan. 2009, p. 449–460 Vol. 191, No. 2
0021-9193/09/$08.00+0 doi:10.1128/JB.01219-08

The pH-Responsive Regulon of HP0244 (FlgS), the Cytoplasmic Histidine Kinase of *Helicobacter pylori*▽

Yi Wen,* Jing Feng, David R. Scott, Elizabeth A Marcus, and George Sachs

The Membrane Biology Laboratory, Department of Physiology, David Geffen School of Medicine at UCLA, and VA Greater Los Angeles Healthcare System, Los Angeles, California 90073

Received 29 August 2008/Accepted 27 October 2008

Helicobacter pylori **colonizes the acidic gastric environment, in contrast to all other neutralophiles, whose acid resistance and tolerance responses allow only gastric transit. This acid adaptation is dependent on regulation of gene expression in response to pH changes in the periplasm and cytoplasm. The cytoplasmic histidine kinase, HP0244, which until now was thought only to regulate flagellar gene expression via its cognate response regulator, HP0703, was found to generate a response to declining medium pH. Although not required for survival at pH 4.5, HP0244 is required for survival at pH 2.5 with 10 mM urea after 30 min. Transcriptional profiling of a HP0244 deletion mutant grown at pH 7.4 confirmed the contribution of HP0244 to σ^{54} activation via HP0703 to coordinate flagellar biosynthesis by a pH-independent regulon that includes 14 flagellar genes. Microarray analysis of cells grown at pH 4.5 without urea revealed an additional 22 genes, including 4 acid acclimation genes (*ureA*, *ureB*, *ureI*, and *amiE*) that are positively regulated by HP0244. Additionally, 86 differentially expressed genes, including 3 acid acclimation genes (*ureF*, *rocF* [arginase], and *ansB* [asparaginase]), were found in cells grown at pH 2.5 with 30 mM urea. Hence, HP0244 has, in addition to the pH-independent flagellar regulon, a pH-dependent regulon, which allows adaptation to a wider range of environmental acid conditions. An acid survival study using an HP0703 mutant and an electrophoretic mobility shift assay with in vitro-phosphorylated HP0703 showed that HP0703 does not contribute to acid survival and does not bind to the promoter regions of several genes in the HP0244 pH-dependent regulon, suggesting that there is a pathway outside the HP0703 regulon which transduces the acid-responsive signal sensed by HP0244.**

A unique feature of the neutralophilic bacterium *Helicobacter pylori* is its ability to survive and grow on the acidic surface of the stomach (21, 34, 37), which is dependent on regulation of bacterial gene expression in response to pH changes in either the periplasm or cytoplasm. Crucial to this feature is the ability of the organism to buffer both the periplasm and cytoplasm. This is mainly achieved through the large quantities of urease expressed by *H. pylori* along with the urea channel, UreI, and a cytoplasmic and periplasmic carbonic anhydrase (7, 20, 35). Efflux of NH_3 into the periplasm and generation of HCO_3^- in the periplasm allow both buffering and neutralization of protons transiting the outer membrane. Periplasmic buffering to a pH of ~6.1 allows this organism to mimic a relatively neutral pH environment in the presence of medium acidity that would otherwise prevent survival or growth. However, the cytoplasmic pH falls progressively in response to increasing environmental acidity, and it seems prudent for the organism also to regulate the cytoplasmic pH by either independent or convergent signaling. Further, there are other genes outside the urease operon that are regulated by environmental acidity that can also contribute to gastric colonization (2, 8, 23, 50).

We call the responses of *H. pylori* that allow survival and growth at an acidic pH acid adaptation in order to contrast these responses with the acid tolerance and resistance responses of other neutralophiles that allow survival but not growth under acid conditions (12). In addition, we designated a group of 12 genes that may contribute to pH homeostasis the acid acclimation group of genes. These genes include, for example, the genes of the urease operon and genes encoding amidases, arginase, asparaginase, and carbonic anhydrases (35).

Two-component systems that consist of a sensor histidine kinase and a cognate response regulator are widespread prokaryotic signal transduction devices that allow regulation of cellular functions in response to changing environmental conditions (14, 26, 39). Besides the chemotaxis proteins CheAY2 (HP0392), CheY1 (HP1067), CheV1 (HP0019), CheV2 (HP0616), and CheV3 (HP0393), *H. pylori* contains only three histidine kinases and five response regulators involved in transcriptional regulation (1, 44).

One of the three complete two-component systems of *H. pylori*, HP1365-HP1364 (CrdRS), has been demonstrated to positively regulate the expression of the copper resistance determinant CrdAB-CzcAB in response to increasing concentrations of copper ions (46). Although the sensor histidine kinase HP1364 (CrdS) has been reported to be required for acid resistance in *H. pylori* strain J99 when it is incubated at pH 5.0 (19), HP1365-HP1364 (CrdRS) was found to be not involved in pH-responsive gene regulation in *H. pylori* strains 26695 and G27 (31).

Another two-component system, HP0166-HP0165 (ArsRS), is known to mediate pH-responsive transcriptional control of the urease gene cluster and of other genes in the acid acclimation group (28–30, 48, 49). These genes generate proteins that are capable of generating a buffer in response to acidification. This two-component system also regulates many other genes (28). The histidine-rich input domain of the sensor his-

* Corresponding author. Mailing address: 11301 Wilshire Blvd., Bldg. 113, Rm. 324, Los Angeles, CA 90073. Phone: (310) 268-4672. Fax: (310) 312-9478. E-mail: ywen@ucla.edu.

▽ Published ahead of print on 31 October 2008.

tidine kinase HP0165 (ArsS) is in the periplasm, which allows a response to pH changes in this compartment.

H. pylori also needs to respond to changes in its cytoplasmic pH that occur at the acidic pH of its gastric habitat. The HP0703-HP0244 (FlgRS) two-component system is part of the regulatory network governing flagellar gene expression (24). The HP0244 histidine kinase (FlgS) is the only cytoplasmic sensor protein in *H. pylori*, based on its domain structure deduced from the genome sequences (1, 44). A previous study (19) showed that HP0244 (FlgS) was not required for survival of *H. pylori* strain J99 under acid conditions when it was exposed to pH 5.0. However, this organism is likely exposed to a considerably lower external pH than pH 5.0 in vivo, as shown by transcriptome analysis of organisms that live in the gastric environment (37). The cytoplasmic location of HP0244 makes it a candidate for a molecule that mounts a response to a decrease in the cytoplasmic pH.

Here, we show that HP0244 is not required for acid survival at pH 4.5 but is required for survival at pH 2.5 even in the presence of 10 mM urea. Deletion of the *H. pylori* cognate response regulator, HP0703, does not affect acid survival in vitro, indicating that a different response element interacts with HP0244 for the acid response. At a medium pH of 4.5, an HP0244 regulon was identified by transcriptome analysis, and this regulon contains 22 genes other than the genes regulated by HP0703, including the acclimation genes *ureA*, *ureB*, *ureI*, and *amiE*. An additional 86 genes were found to be regulated by HP0244 at a medium pH of 2.5 with 30 mM urea. These genes include additional members of the acid acclimation group, *ureF*, *rocF* (arginase), and *ansB* (asparaginase). These results indicate that HP0244 is a cytoplasmic pH-responsive histidine kinase, which adds to the role of this protein as a regulator of flagellar gene expression.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *H. pylori* strain 26695 was obtained from the American Type Culture Collection. HP0244 gene-deficient mutant 26695/ΔHP0244::km and HP0703 gene-deficient mutant 26695/ΔHP0703::km were constructed by allelic exchange using a kanamycin resistance gene as described below. Primary plate cultures of *H. pylori* were grown from glycerol stocks on blood agar for 2 to 3 days in a microaerobic environment (5% O_2, 10% CO_2, 85% N_2) at 37°C. In preparation for an experiment, bacteria were scraped from the plates, suspended in 1 mM phosphate HP buffer (138 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 10 mM glucose, 1 mM glutamine; pH 7.0), transferred to fresh plates, and incubated for 24 h. For exposure to experimental low-pH conditions, overnight cultures of *H. pylori* strain 26695 on Trypticase soy agar (TSA) plates supplemented with 5% sheep blood were suspended in brain heart infusion (BHI) medium (Difco) to obtain an optical density at 600 nm of about 0.20 to 0.25. The pH of BHI medium was adjusted to 7.4, 4.5, or 2.5 using concentrated HCl, and this was followed by filtration to remove any precipitate. *H. pylori* was then incubated in the presence of urea (at the concentrations indicated below) with shaking (120 rpm) under microaerobic conditions at 37°C for 30 min. *Escherichia coli* strains were grown in Luria-Bertani broth. When necessary, antibiotics were added at the following final concentrations: ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; and chloramphenicol, 30 μg/ml. The motility of *H. pylori* strains was assayed by stab inoculating bacteria with a pipette tip onto 0.3% agar plates containing BHI medium and Dent's antibiotic supplement.

Construction of *H. pylori* mutant strains 26695/ΔHP0244::km and 26695/ΔHP0703::km by allelic exchange mutagenesis. The isogenic HP0244 gene-deficient mutant 26695/HP0244::km was obtained by transforming *H. pylori* strain 26695 with a pBluescript II vector (Stratagene) carrying the Tn*903* kanamycin resistance gene (41) flanked by a 369-bp fragment comprising the 313-bp 3′ region of the HP0245 gene and the 56-bp 5′ region of the HP0244 gene and by a 407-bp fragment comprising the 199-bp intergenic region between the HP0244 and HP0243 genes and the 208-bp 5′ region of the HP0243 gene (Fig. 1A). The DNA fragments were obtained by performing PCR with *H. pylori* chromosomal DNA and primers HP0245-5′P(1229-1248)-KpnI and HP0244-3′P(1597-1577)-EcoRI and primers HP0243-5′P(2681-2702)-BamHI and HP0243-3′P(3087-3064)-SacI (Table 1), respectively. In the resulting mutants almost the entire HP0244 gene coding sequence was replaced by the kanamycin gene. Measurement of gene expression in the deletion mutant identified genes that were downregulated in the mutant and hence positively regulated in the wild-type strain.

To construct an *H. pylori* HP0703 gene-deficient strain (26695/ΔHP0703::km), a pBluescript II vector (Stratagene) carrying the kanamycin resistance cassette flanked by a 465-bp fragment comprising 277 bp of the 3′ region of the HP0702 open reading frame (ORF) and 191 bp of the 5′ region of the HP0703 ORF and by a 484-bp fragment comprising 331 bp of the 3′ region and 154 bp of the intergenic region downstream of the HP0703 ORF (Fig. 1C) was introduced into *H. pylori* strain 26695 by natural transformation. The DNA fragments were obtained by PCR amplification of chromosomal DNA of *H. pylori* 26695 with primers HP0702-5′P(4424-4448)-KpnI and HP0703-3′P(4888-4864)-EcoRI and primers HP0703-5′P(5512-5534)-BamHI and HP0703-3′P(5995-5971)-SacI (Table 1), respectively. In the resulting strain (26695/ΔHP0703::km) the center part (625 bp) of the HP0703 gene was replaced by a kanamycin resistance cassette. The kanamycin-selected mutant strains were confirmed by PCR.

Reintroduction of the HP0244 ORF. To restore the HP0244 ORF in the HP0246-HP0244 operon of strain 26695/ΔHP0244::km, a pBluescript II vector carrying a chloramphenicol resistance cassette (47) flanked by a 1,452-bp fragment comprising a 313-bp 3′ region of the HP0245 gene and the complete 1,146-bp HP0244 ORF and by a 407-bp fragment comprising the 199-bp intergenic region between the HP0244 and HP0243 genes and the 208-bp 5′ region of the HP0243 gene (Fig. 1B) was introduced into *H. pylori* strain 26695/ΔHP0244::km by natural transformation. The DNA fragments were obtained by PCR amplification of chromosomal DNA of *H. pylori* 26695 using primers HP0245-5′P(1229-1248)-KpnI and HP0244-RP(2569-2680)-EcoRI and primers HP0243-5′P(2681-2702)-BamHI and HP0243-3′P(3087-3064)-SacI (Table 1), respectively. In the resulting strain (26695/rHP0244-cm) the disrupted HP0244 ORF and kanamycin resistance cassette were replaced by a complete HP0244 ORF and a chloramphenicol resistance cassette. The chloramphenicol-resistant and kanamycin-sensitive transformants were examined by PCR to confirm that the kanamycin resistance cassette was replaced by the HP0244 ORF and that there was concomitant integration of the chloramphenicol resistance cassette.

Acid survival studies. *H. pylori* wild-type strain 26695, the HP0244 gene-deficient mutant, the strain with the HP0244 gene restored, and the HP0703 gene-deficient mutant were grown overnight on BHI agar plates with appropriate antibiotics. The bacteria were removed from the plates and then resuspended at a concentration of 1×10^9 cells/ml in 10 ml of BHI medium at pH 7.4, at pH 4.5 without urea, or at pH 2.5 with 10, 20, or 30 mM urea and incubated for 30 min in a microaerobic environment at 37°C. The higher concentrations of urea under the latter conditions were used to provide more buffering capacity at the acidic pH to retain mRNA integrity for microarray studies. Tenfold serial dilutions of the bacterial suspensions were spread on TSA plates supplemented with 5% sheep blood and incubated for 3 to 5 days in a microaerobic atmosphere at 37°C before quantitation of bacterial colonies based on counts on petri dishes and calculation of the number of CFU. The survival under acidic pH conditions was determined by comparison to pH 7.4 controls. All experiments were performed in duplicate, and at least four independent experiments were performed for each condition.

RNA preparation. Total RNA was isolated from *H. pylori* strains using TRIzol reagent (Invitrogen, California) combined with RNeasy columns (Qiagen, California). Each bacterial pellet was resuspended in 500 μl of TRIzol reagent (Invitrogen) and lysed at room temperature for 5 min before 100 μl of chloroform was added. After centrifugation at 12,000 × *g* for 10 min at 4°C, the supernatant was mixed with 250 μl ethanol and applied to an RNeasy spin column (Qiagen), and then the RNA was purified by following the manufacturer's instructions (beginning with application to the column). The RNA concentration was determined by determining the absorbance at 260 nm, and the quality was evaluated by capillary electrophoresis using an Agilent 2100 bioanalyzer with an RNA 6000 Nano assay kit (Agilent Technologies).

Fluorescent cDNA labeling and microarray analysis. For each comparative array hybridization, labeled cDNA was synthesized by reverse transcription from RNAs of the wild-type strain after treatment at pH 7.4, at pH 4.5 without urea, or at pH 2.5 (30 mM urea) with Cy5-dCTP and from RNAs of the HP0244 gene-deficient mutant strain (after treatment under the different pH conditions) with Cy3-dCTP, using a Superscript II reverse transcription kit (Invitrogen). For each reverse transcription reaction, 1 μg of random primers (Invitrogen) and 40 μg of *H. pylori* RNA were mixed in a 12.5-μl (final volume) mixture, heated to

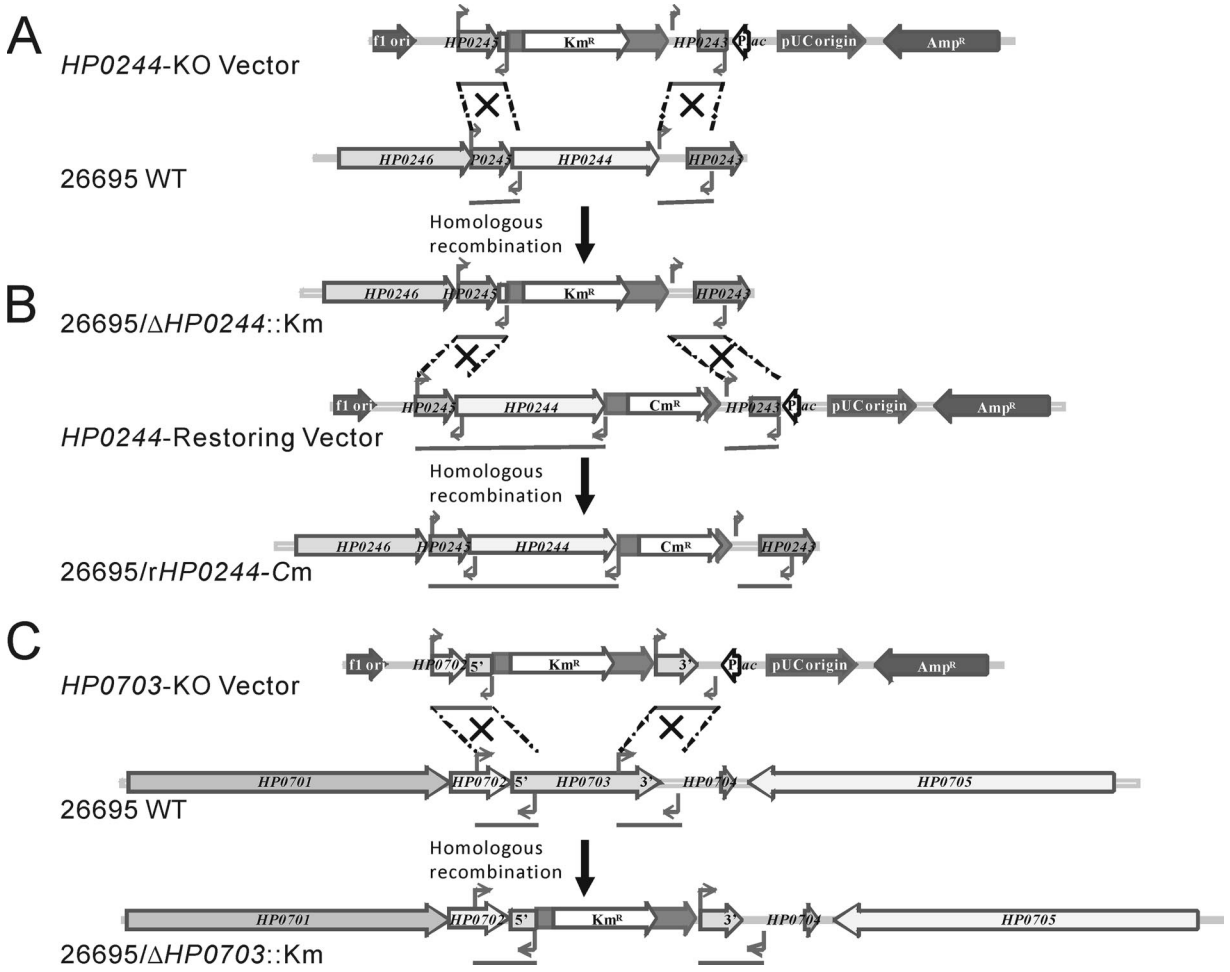

FIG. 1. Strategy for construction of *H. pylori* strains 26695/ΔHP0244::km (A), 26695/rHP0244-cm (B), and 26695/ΔHP0703::km (C) by allelic replacement mutagenesis. The bent arrows indicate primer pairs that were used to amplify flanking DNA fragments (indicated by bars) of target genes. The major parts of the HP0244 and HP0703 ORFs were replaced by a kanamycin resistance cassette (Km^R) in 26695/ΔHP0244::km and 26695/ΔHP0703::km, while the intact HP0244 ORF was reintroduced with a chloramphenicol resistance cassette (Cm^R) in 26695/rHP0244-cm. WT, wild type; KO, knockout.

70°C for 10 min, and chilled on ice. Fluorescent cDNA probe synthesis was performed at 42°C for 3 h using a 25-μl reaction mixture containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM dATP, 0.5 mM dGTP, 0.5 mM dTTP, 0.2 mM dCTP, 0.1 mM Cy3- or Cy5-conjugated dCTP (Amersham), 40 U RNasin (Promega), and 200 U Superscript II reverse transcriptase (Invitrogen.). Following reverse transcription, the RNA template was degraded by incubating it for 40 min at 65°C in 0.27 M NaOH, followed by neutralization with Tris-HCl buffer. Labeled cDNA was purified and concentrated prior to hybridization using Microcon 30 concentrators (Amicon).

H. pylori microarrays containing 1,534 predicted ORFs of *H. pylori* strain 26695 (50) were hybridized in a 20-μl (final volume) mixture containing 3× SSC, 0.8 mg/ml salmon sperm DNA, and 0.2% sodium dodecyl sulfate (SDS) (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). Prior to hybridization, combined Cy3- and Cy5-labeled probes (representing mutant and wild-type strains) were heated to 95°C for 2 min and pipetted directly onto microarray slides. A glass coverslip (22 by 22 mm) was applied to each slide, and the arrays were hybridized overnight at 65°C in a humidified hybridization chamber. Following hybridization, the slides were washed for 5 min in 0.2× SSC with 0.1% SDS and then twice (3 min each) in 0.2× SSC and rinsed in 0.05× SSC. To remove the residual salts, the slides were spun at 500 rpm for 5 min prior to scanning.

Signal intensities for each spot were determined using Phoretix Array software (Nonlinear Dynamic). Following subtraction of the background value, signal intensities were expressed as percentages of the total signal for all spots as a means of normalization. The values were used to determine the ratio of wild-type signals to HP0244 gene-deficient mutant signals. A threshold minimum acceptable signal (two standard deviations above the background intensity) was used to eliminate expression ratios that were extremely high or low due to signals that were too low for wild-type or mutant samples. A *t* test was used to determine the consistency of ratios across replicate hybridizations. Only genes whose ratios showed that there was a ≥2-fold change (either increase or decrease) and that had a 95% confidence interval as determined by the *t* test were considered significantly regulated genes.

Northern blot analysis. Fifteen-microgram portions of total RNA from different *H. pylori* samples were fractionated on 1% agarose-formaldehyde gels and transferred to NYTRAN membranes (Schleicher & Schuell, Keene, NH). The RNA on the gels was visualized by ethidium bromide staining and photographed before it was transferred to NYTRAN membranes.

DNA fragments of the *ureA* (HP0073), *ureI* (HP0071), and *amiE* (HP0294) genes were PCR amplified from genomic DNA of *H. pylori* 26695 with the primer pairs pHP0072-0073R_5′/HP0073_3′(580-603), HP0071_5′(249-272)/HP0071_3′(821-840), and HP0294_5′(510-533)/HP0294_3′(922-901) (Table 1) and used as probes. The probes were radiolabeled with [α-^{32}P]dCTP using a random primer labeling kit (Stratagene, La Jolla, CA) to obtain specific activities of about 1×10^8 to 10×10^8 cpm/μg. Each blot was hybridized (9) with radiolabeled probe overnight at 65°C in a buffer containing 0.45 M sodium phosphate (pH 7.2), 7% SDS, 1% bovine serum albumin, and 20 mM EDTA. The hybridized blot was washed with 0.1× SSC–0.1% SDS at 65°C and autoradiographed using a 445 SI PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

TABLE 1. Oligonucleotide primers used in this study

Primer	Sequence (5′ to 3′)[a]	Restriction recognition site	Strand	Position[b]
HP0245-5′P(1229-1248)	**ATTGGGTACC**AAAGCCATGTTAGAGCAGTA	KpnI	−	254337–254356
HP0244-3′P(1597-1577)	**GCAGGAATCC**AAGCCTTATCAGAGTGTTTCA	EcoRI	+	253988–254008
HP0243-5′P(2681-2702)	**TAGTGGATCC**AACTATCCCCCTTAAAAATGAG	BamHI	−	252883–252904
HP0243-3′P(3087-3064)	**TGTTGAGCTC**GATAAAGTGACTAAGGGGTGATGC	SacI	+	252498–252521
HP0702-5′P(4424-4448)	**ATTGGGTACC**TCATTGCTCCATCATCACTCGTAAC	KpnI	+	755192–755216
HP0703-3′P(4888-4864)	**GCAGGAATCC**GCGTAAAAATTCCAAGCCGTCCATA	EcoRI	−	755632–755656
HP0703-5′P(5512-5534)	**TAGTGGATCC**CATTTCCGCTACCAACGCCAACA	BamHI	+	756280–756302
HP0703-3′P(5995-5971)	**TGTTGAGCTC**AACTCTCCCTCAAGCTAATCCTACT	SacI	−	756739–756763
HP0244-RP(2569-2680)	**GCAGGAATCC**ATCCAAATTAAGAAGCGTTAAG	EcoRI	+	252905–252926
HP0073_3′(580-603)	ACATCGCTTCAATACCCACTTCAT		+	77698–77721
HP0071_5′(249-272)	GGCAATGCTAGGACTTGTATTGTT		−	75315–75338
HP0071_3′(821-840)	TTATCCAACACTGGGTGTGA		+	74747–74766
HP0294_5′(510-533)	ACACGAGCAAGCCAAAAAGAATCC		+	311340–311363
HP0294_3′(922-901)	CGCCCAAAGTATGCCCGTCAAA		−	311731–311752
HP0703-FP(4693-4720)	**CACC**ATGAAAATCGCCATTGTAGAAGAT		+	755465–755488
HP0703-RP(5820-5844)	CCCTACCTTTCCAAAAACAAATCTT		−	756588–756612
pHP0073-0072R_5′	CATTATCACTCCAATTTTAA		−	78188–78207
pHP0073-0072R_3′	TTATTCTCCTATTCTTAAAG		+	77958–77977
pHP0067-0071R_5′	GATTTTTTAGGAGCAACGCT		−	75507–75526
pHP0067-0071R_3′	GCCTTTTCCTTCCAAACAAA		+	75336–75355
pHP1559-HP1558_5′	TTTTTGGTTTTTGAATAGG		−	1641552–1641570
pHP1559-HP1558_3′	TAGAAAAATCCATAATAAAACC		+	1641364–1641385

[a] Nucleotides in light type were derived from the genome sequence of *H. pylori* 26695 (44). Nucleotides introduced for cloning purposes are indicated by bold type, and restriction recognition sites are underlined.
[b] Nucleotide positions in the genome sequence of *H. pylori* 26695 (44).

Construction of HP0703-His$_6$ expression plasmid. The DNA fragment coding for response regulator HP0703 was amplified by PCR using genomic DNA from *H. pylori* strain 26695 as the template and primers HP0703-FP(4693-4720) and HP0703-RP(5820-5844). The 5′ primer HP0703-FP(4693-4720) contained a 4-nucleotide sequence (CACC) immediately 5′ of the ATG starting codon to facilitate directional cloning into the pET100/D-TOPO vector (Invitrogen), which allowed expression of recombinant HP0703 with an N-terminal tag containing the Xpress epitope and a six-His tag. The nucleotide sequences of the cloned PCR products were verified by sequencing both strands.

Overproduction and purification of HP0703-His$_6$. HP0703-His$_6$ recombinant protein was expressed in *E. coli* BL21 Star(DE3) containing plasmid pET100/D-TOPO-HP0703. Bacteria were grown in 50 ml of Luria-Bertani broth at 37°C to an optical density at 600 nm of 0.5. Subsequently, isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 1 mM, and the bacteria were incubated for 2 h. Cells were harvested and suspended in 8 ml of native binding buffer (50 mM NaH_2PO_4, 500 mM NaCl, 10 mM Tris-HCl; pH 8.0) with 10 mM imidazole and 1 mg/ml lysozyme. After incubation on ice for 30 min, the bacteria were disrupted by sonication. The cell debris was pelleted by centrifugation, and 8 ml of the supernatant was added to a purification column containing 1.5 ml of 50% Ni-nitrilotriacetic acid resin (Invitrogen) and incubated for 3 h at room temperature on an orbital shaker. The column was washed four times with 8 ml of native wash buffer (50 mM NaH_2PO_4, 500 mM NaCl, 10 mM Tris-HCl [pH 8.0], 20 mM imidazole). His-tagged proteins were eluted with 8 ml of native elution buffer (50 mM NaH_2PO_4, 500 mM NaCl, 10 mM Tris-HCl [pH 8.0], 250 mM imidazole). An aliquot (10 μl) of each eluted fraction was analyzed by SDS-polyacrylamide gel electrophoresis, and the fractions with purified proteins were concentrated and desalted using Amicon Ultra-4 centrifugal filter devices (Millipore).

EMSA. DNA fragments corresponding to the promoter regions of the *ureAB*, *ureI*, and *flgBC* genes were generated by performing PCR with primer pairs pHP0073-0072R_5′/pHP0073-0072R_3′, pHP0067-0071R_5′/pHP0067-0071R_3′, and pHP1559-HP1558_5′/pHP1559-HP1558_3′ (Table 1) and used as probes. These probes were 5′ end labeled radioactively by using T4 polynucleotide kinase and [γ-^{33}P]ATP (50 μCi). A sample of recombinant HP0703-His$_6$ was phosphorylated in vitro in a buffer containing 20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 25 mM KCl, 1 mM dithiothreitol, and 10 mM carbamylphosphate for 60 min at 25°C. Binding of HP0703-His$_6$~P or HP0166-His$_6$~P (49) (as a control) to DNA was carried out by using a 10-μl reaction mixture containing 10^4 cpm of ^{33}P-labeled DNA, 1 μg of poly(dI-dC) (Sigma), 25 mM $NaPO_4$ (pH 7), 150 mM NaCl, 0.1 mM $MgSO_4$, and 1 mM dithiothreitol. The DNA binding reaction was initiated by addition of HP0703-His$_6$, and the mixture was incubated at room temperature for 20 min. Radiolabeled PCR-generated probes corresponding to the promoter-regulatory regions for the *ureAB*, *ureI*, and *flgBC* genes were incubated with different amounts of purified HP0703-His$_6$. Cold competitor chase experiments performed with a 50-fold excess of unlabeled probe as a specific competitor were used to demonstrate the specificity of HP0703 binding. Samples were then loaded directly onto a 6% DNA retardation polyacrylamide gel (Invitrogen). Electrophoresis was carried out for 1 h at room temperature (14 V/cm), and the gels were then dried and analyzed by autoradiography. Each electrophoretic mobility shift assay (EMSA) experiment was repeated at least three times.

Transcriptome data accession number. All transcriptome data described here have been deposited in the NCBI Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/) under GEO series accession number GSE12900.

RESULTS

HP0244 (FlgS) is not required for acid survival of *H. pylori* at pH 4.5 but is required at pH 2.5. To explore a possible role of HP0244 in the adaptation of *H. pylori* to acid, the 26695 wild-type strain and the HP0244 gene-deficient mutant 26695/ΔHP0244::km were used in an acid survival analysis (Fig. 2). After 30 min of exposure to pH 4.5 without urea (the final pH was 4.64 ± 0.03), there was no significant difference in survival between the 26695/ΔHP0244::km mutant and the wild-type control at pH 7.4. This showed that HP0244 was not required for acid survival at a medium pH of 4.5 without urea, which resulted in different acidic pH values in the periplasm and cytoplasm at 100 s [pH 4.5 and 5.3, respectively, as determined by using 2′,7′-bis-(2-carboxyethyl)-5(and 6)-carboxyfluorescein (BCECF) fluorescence] (50) but the same pH in the cytoplasm and periplasm after 30 min (pH ~4.5). However, at a medium pH of 2.5 with 10 mM urea (the final pH was 3.07 ± 0.16 after 30 min), a significant ~7-log$_{10}$ decrease in survival was observed for strain 26695/ΔHP0244::km.

To verify that the observed defect in acid survival was due specifically to mutation of HP0244, we reversed the mutation by replacing the mutated locus with an intact copy of the HP0244

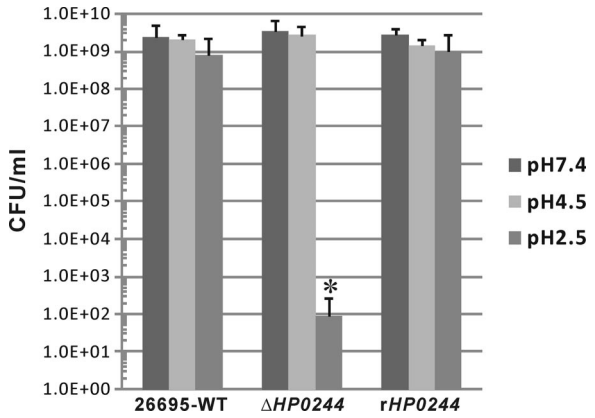

FIG. 2. Acid survival analysis showing that HP0244 (FlgS) is not required for acid survival of *H. pylori* at pH 4.5 but is required at pH 2.5. Cells of *H. pylori* wild-type strain 26695 (26695-WT), mutant strain 26695/ΔHP0244::km (Δ*HP0244*), and reversion strain 26695/rHP0244-cm (r*HP0244*) were grown overnight on agar plates, harvested, and resuspended at a concentration of 1×10^9 cells/ml at pH 7.4, pH 4.5 without urea, or pH 2.5 with 10 mM urea. After incubation at 37°C for 30 min, serial 10-fold dilutions of each bacterial cell suspension were spread on TSA plates and incubated for 3 to 5 days at 37°C, which was followed by calculation of the number of CFU. The data are averages of four separate experiments (*, $P < 0.005$). The error bars indicate the standard deviations.

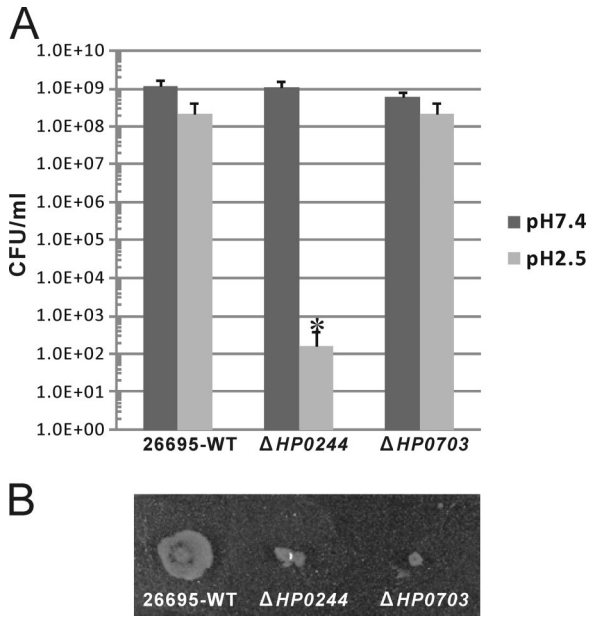

FIG. 3. Loss of motility of *H. pylori* does not correlate with the acid survival defect. (A) Acid survival analysis with *H. pylori* wild-type strain 26695 (26695-WT) and mutant strains 26695/ΔHP0244::km (Δ*HP0244*) and 26695/ΔHP0703::km (Δ*HP0703*) at pH 2.5 with 10 mM urea. *, $P < 0.005$. The error bars indicate the standard deviations. (B) Bacterial motility assay. The indicated strains were stabbed into semisolid agar medium and incubated at 37°C for 72 h. Equivalent nonmotile phenotypes were observed for mutant strains 26695/ΔHP0244::km and 26695/ΔHP0703::km.

ORF and performed acid survival studies with reversion strain 26695/rHP0244-cm. The results (Fig. 2) showed that reintroduction of the HP0244 ORF into *H. pylori* 26696/ΔHP0244::km fully restored acid survival at pH 2.5. Therefore, HP0244 is involved in acid survival. This is the first time that HP0244 has been found to be implicated in the acid responses of *H. pylori*.

The acid survival defect of the 26695/ΔHP0244::km mutant is not due to the nonmotile phenotype. The HP0244-HP0703 (FlgSR) two-component system has been demonstrated to be required for motility and expression of σ^{54}-dependent flagellar genes (6, 24, 38). To determine if the acid survival defect of the 26695/ΔHP0244::km mutant is due to the nonmotile phenotype, a mutant strain (26695/ΔHP0703::km) with HP0703 (a response regulator of HP0244 [24]) deleted was generated by allelic exchange mutagenesis of the *H. pylori* 26695 strain. In vitro acid survival analysis of this mutant did not reveal any significant defect in acid survival at pH 2.5 (Fig. 3A), while mutants 26695/ΔHP0244::km and 26695/ΔHP0703::km showed similar nonmotile phenotypes in a motility assay (Fig. 3B). These results demonstrate that the acid survival defect of the 26695/ΔHP0244::km mutant is not due to a lack of motility.

Two HP0244 regulons (a pH-independent regulon and a pH-dependent regulon) identified by transcriptional profiling. To define the HP0244 acid-responsive regulon in *H. pylori*, RNA was isolated from the 26695 wild-type strain and the HP0244 gene-deficient mutant 26695/ΔHP0244::km subjected to three different pH conditions (pH 7.4, pH 4.5 without urea, and pH 2.5 with 30 mM urea) for 30 min. The higher urea concentration at pH 2.5 was necessary to improve the quality of the RNA so that it was suitable for transcriptome analysis following exposure to pH 2.5. In contrast to the results obtained with 10 mM urea, where the level of survival decreased by ~7 $\log_{10}$, in the presence of 30 mM urea (the final pH after 30 min was 4.24 ± 0.04) the level of survival was about ~1 $\log_{10}$ less than that of the wild type (data not shown). These results indicated that under these conditions the gene regulation by HP0244 was different from that observed at pH 4.5 due to the lower pH of the medium. Fluorescently labeled cDNAs from the wild-type strain (labeled with Cy3) and 26695/ΔHP0244::km (labeled with Cy5) were prepared from the RNAs, and the differentially labeled cDNA pairs derived from the *H. pylori* wild-type strain and HP0244 mutant under each pH condition were hybridized to glass slide DNA microarrays containing 1,534 ORFs of *H. pylori* strain 26695 (50).

The genes that are regulated by HP0244 were classified into three groups. One group was always independent of pH and included the genes responsible for regulation of flagellar gene expression, the genes in the second group responded to a medium pH of 4.5, and the genes in the third group responded only to a medium pH of 2.5.

Seventeen genes in the first group were determined to be differentially expressed in the HP0244 mutant under all three pH conditions using signal ratio cutoff values of <0.5 and >2.0 (Table 2). All of these genes were regulated by HP0244 with a signal ratio (wild type/ΔHP0244) of >2.0. No genes negatively regulated by HP0244 with a wild type/ΔHP0244 ratio of <0.5 were found. Thirteen of these genes (Table 2) are in the HP0244 regulon identified by Niehus et al., who used *H. pylori* strain N6 at neutral pH in their study (24). They include nine genes (HP0115 [*flaB*], HP1119 [*flgK*], HP0295 [*fla*], HP0870 [*flgE*], HP0869 [*hypA*], HP1120, HP1233, HP0114, and HP0906 genes) that are class 2 flagellar genes (middle flagellar structural genes) and are governed by sigma factor σ^{54} and four

TABLE 2. pH-independent HP0244 regulon

ORF	Wild-type strain/ΔHP0244 strain ratio[a]			Description
	pH 7.4	pH 4.5	pH 2.5	
HP0366[b]	3.30	3.11	3.09	Spore coat polysaccharide biosynthesis protein C
HP0115[b,c,d]	4.74	4.89	4.85	Flagellin B (*flaB*)
HP1119[b]	3.47	5.10	5.85	Flagellar hook-associated protein 1 (*flgK*)
HP0295[b,c,d]	3.20	3.43	3.32	Flagellin B homolog (*fla*)
HP1559[b,c,d]	2.80	2.83	2.75	Flagellar basal body rod protein (*flgB*)
HP0907[b]	2.45	2.18	2.43	Hook assembly protein, flagella (*flgD*)
HP0870[b,d]	2.57	2.80	2.93	Flagellar hook (*flgE*)
HP0869[b,c,d]	2.53	2.75	3.20	Hydrogenase expression/formation protein (*hypA*)
HP1120[b]	5.08	4.51	4.29	*H. pylori* predicted coding region HP1120
HP0367[b,c,d]	3.11	3.37	3.88	*H. pylori* predicted coding region HP0367
HP1233[b,d]	3.26	2.76	2.72	*H. pylori* predicted coding region HP1233
HP0114[b]	2.57	2.83	2.99	*H. pylori* predicted coding region HP0114
HP0906[b]	2.22	2.13	2.63	*H. pylori* predicted coding region HP0906
HP0695[c]	2.53	3.02	3.39	Hydantoin utilization protein A (*hyuA*)
HP0696	4.51	4.09	4.71	*N*-Methylhydantoinase
HP0697[c]	9.36	9.41	7.46	*H. pylori* predicted coding region HP0697
HP1111	3.64	2.07	4.73	Pyruvate ferredoxin oxidoreductase, beta subunit

[a] Average ratios based on three independent microarray hybridizations with separate RNA preparations for each pH condition.
[b] Flagellar gene identical to an HP0244 regulon gene identified by Niehus et al. (24).
[c] Acid-responsive gene identified by Wen et al. (50).
[d] Acid-responsive gene identified by Merrell et al. (23).

genes (HP0366, HP0367, HP1559 [*flgB*], and HP0907 [*flgD*] genes) that belong to the intermediate class of structural and regulatory flagellar genes which are controlled by more than one promoter. In addition, an operon consisting of three genes (HP0695, HP0696, and HP0697 genes) that may be involved in acetone metabolism (27) and the HP1111 gene encoding a β subunit of pyruvate ferredoxin oxidoreductase were also identified as part of the HP0244 regulon under all three pH conditions. The consistent upregulation by HP0244 at pH 7.4, 4.5, and 2.5 shows that a pH-independent HP0244 regulon is responsible for regulation of flagellar gene expression.

Twenty-two genes in the second group were found to be regulated by HP0244 in response to a medium pH of 4.5 without urea. These findings reflect a pH-dependent HP0244 regulon involved in the acid adaptation of *H. pylori*. As shown in Table 3, four of the acid acclimation genes (29, 33, 35) (*ureA*, *ureB*, *ureI*, and *amiE*) were in this HP0244 pH 4.5 regulon. However, data for the levels of expression of all of these genes except *ureI* did not reveal further upregulation at pH 2.5.

Eighty-six genes in the third group were found to be positively regulated by HP0244 in response to a lower medium pH (pH 2.5 with 30 mM urea) (Table 4), expanding the pH-dependent HP0244 regulon to total of 108 genes. Among the genes regulated at pH 2.5, three genes belonging to the acid acclimation group (*ureF*, *ansB* [asparaginase], and *rocF* [arginase]) were different in the deletion mutant (50). A group of oxidative stress response genes (HP1563 [*ahpC*], HP0825 [*trxR*], and HP0874 [*kapA*] genes) (3, 13) are also members of the HP0244 regulon (Table 4). This group also contained 14 genes involved in outer membrane protein synthesis, as well as the HP0243 gene [*napA*], another stress response gene (10), suggesting that HP0244 may be involved in regulation of a stress response induced by low medium pH (pH ~2.5).

The results of transcriptional profiling of the HP0244 gene-deficient *H. pylori* mutant under different pH conditions support the concept that HP0244 is one of the factors that contributes to σ^{54} activation to coordinate flagellar biosynthesis. This unusual NtrB-like histidine kinase lacking transmembrane domains has been shown to be required for acid survival in *H. pylori* exposed to pH 2.5. Together with the transcriptional profile results for pH 4.5 and 2.5, the data suggest that HP0244 may also play an important role in the acid regulatory network by sensing and responding to a drastic decrease in the medium pH.

Validation of the microarray data. To validate results for the HP0244 regulon that is involved in the acid response (pH-dependent HP0244 regulon), Northern blot analysis was performed with equal amounts of total RNAs extracted from *H. pylori* strain 26695 and HP0244 gene-deficient mutant 26695/ΔHP0244::km which had been exposed to either neutral pH (pH 7.4) or a low pH (pH 4.5 or 2.5) for 30 min. Probes corresponding to the acid acclimation genes found in the pH-dependent HP0244 regulon, *ureI*, *amiE*, and *ureAB*, were used for the Northern blot analysis.

The Northern blot analysis revealed double bands for the HP0071 (*ureI*) transcript at 1.0 and 0.8 kb (corresponding to *ureIE* and *ureI*, respectively) (Fig. 4A). In the 26695 wild-type strain, transcription of *ureI* was induced at pH 4.5 and 2.5 (the transcription was about two- to threefold greater than that at pH 7.4). By contrast, no significant change in the level of transcription of *ureI* in the HP0244 gene-deficient mutant was observed when the bacteria were exposed to pH 7.4 and low-pH conditions (pH 4.5 or 2.5) (Fig. 4A).

A single band (~1.2 kb) was obtained for the HP0294 (*amiE*) transcript (Fig. 4B). As observed for *ureI*, the level of transcription of *amiE* in the 26695 wild-type strain increased about twofold when the bacteria were exposed to low-pH conditions. A relatively small increase in the level of transcription of *amiE* was observed for the HP0244 gene-deficient mutant in response to pH 4.5, suggesting that some regulatory system other than HP0244 may be also responsible for the upregulation of *amiE* in response to low pH.

TABLE 3. pH 4.5-dependent HP0244 regulon

Functional category	ORF	Wild-type strain/ΔHP0244 strain ratio[a]			Description
		pH 7.4	pH 4.5	pH 2.5	
Acid acclimation	HP0071[b,c,e]	1.46	2.10	3.01	Urease accessory protein (*ureI*)
	HP0072[c,e]	1.40	3.81	2.93	Urease beta subunit (urea amidohydrolase) (*ureB*)
	HP0073[c,d,e]	1.31	3.23	2.80	Urease alpha subunit (*ureA*)
	HP0294[b,c,d,e]	1.53	2.43	2.83	Aliphatic amidase (*amiE*)
Energy metabolism	HP1132[d]	1.73	2.17	3.72	ATP synthase F1, subunit beta (*atpD*)
	HP0589[b]	1.69	2.50	2.82	Ferredoxin oxidoreductase, alpha subunit
	HP0590[b]	1.73	2.61	2.70	Ferredoxin oxidoreductase, beta subunit
	HP0692[b]	1.66	2.05	2.96	3-Oxoadipate coenzyme A transferase subunit B (*yxjE*)
	HP1346	1.59	2.55	2.77	Glyceraldehyde-3-phosphate dehydrogenase (*gap*)
Oxidative stress	HP0824[b,e]	1.62	2.13	3.07	Thioredoxin (*trx1*)
	HP0875[b,e]	1.25	2.60	2.86	Catalase (*katA*)
Cellular processes	HP1496	1.64	2.07	2.59	General stress protein (*ctc*)
	HP0599	1.43	1.96	2.78	Hemolysin secretion protein precursor (*hylB*)
	HP0547[c]	1.84	2.23	4.16	*cag* pathogenicity island protein (*cag26*)
Protein fate and translation	HP0074	1.63	3.03	2.91	Lipoprotein signal peptidase (*lspA*)
	HP1205	1.77	2.37	3.21	Translation elongation factor EF-Tu (*tufB*)
	HP1153	1.27	3.27	2.83	Valyl-tRNA synthetase (*valS*)
	HP1197	1.41	2.15	2.69	Ribosomal protein S12 (*rps12*)
Transport	HP1169	1.17	2.30	2.73	Glutamine ABC transporter (*glnP*)
Biosynthesis	HP1118	1.67	1.98	2.93	Gamma-glutamyltranspeptidase (*ggt*)
Unknown	HP1083	1.29	2.09	2.41	*H. pylori* predicted coding region HP1083
	HP0449	1.47	2.84	3.06	*H. pylori* predicted coding region HP0449

[a] Average ratios based on three independent microarray hybridizations with separate RNA preparations for each pH condition.
[b] Acid responsive gene identified by Wen et al. (50).
[c] Acid responsive gene identified by Merrell et al. (23).
[d] Acid responsive gene identified by Bury-Mone et al. (8).
[e] HP0165 regulon gene identified by Pflock et al. (28).

A major band at 2.7 kb for HP0073-HP0072 (*ureAB*) obtained with a *ureA* probe was found to be upregulated about three- to fourfold in the wild-type strain in response to low-pH conditions (pH 4.5 and 2.5) compared to the results for bacteria treated at pH 7.4. However, no significant change in the level of transcription of the 2.7-kb *ureAB* gene was observed for the HP0244 gene-deficient mutant exposed to low-pH conditions (Fig. 4C). This confirms that the *ureAB* genes are upregulated by HP0244 in response to low pH.

The cognate response regulator HP0703 (FlgR) is not directly involved in the acid-induced transcriptional regulation of the acid acclimation genes by HP0244. Although regulation of the *ureAB* genes by HP0244 in response to low pH was confirmed by Northern blot analysis, the observed regulation may have been a result of transcriptional control directly regulated by HP0703, or regulation may have occurred via a different response pathway. To test if the response regulator HP0703 is involved in the acid-induced transcriptional regulation of the acid acclimation genes *ureAB* and *ureI*, the HP0703-His_6 protein was overexpressed in *E. coli* and purified for use in EMSA with radioactively labeled probes for the promoters of both *ureAB* and *ureI*. A probe for the promoter of the HP1559 and HP1558 genes (*flgBC*) was used as a positive control.

The absence of a gel shift in EMSA showed that there was no direct binding of phosphorylated HP0703 to either the *ureAB* or *ureI* promoter probe (Fig. 5A and B). As a control, a 206-bp probe for the promoter of the *flgBC* genes, which belong to the intermediate class of flagellar genes (24) and were shown previously to be under the control of HP0703 (38), was found to interact directly with in vitro-phosphorylated HP0703 by a gel shift analysis with 30 pmol of HP0703-His_6~P (Fig. 5C). In another control, EMSA with HP0166 showed that there was a direct interaction between HP0166-His_6 and the *ureAB* promoter (Fig. 5D), confirming that the *ureAB* promoter is directly regulated by HP0166.

These results show that although HP0703 regulates the flagellar genes in tandem with HP0244, it does not regulate the acid-responsive expression of the *ureAB* and *ureI* genes.

DISCUSSION

H. pylori encounters a wide range of acidic pH conditions (pHs between ~1.0 and ~4.5) over a 24-h period in the human stomach (43). In order to colonize the human stomach, *H. pylori* has evolved acid adaptation mechanisms (35) that regulate gene expression and cellular functions in response to pH changes in both the periplasm and cytoplasm. There is a group of genes that can contribute to pH regulation under different conditions, which we have termed acid acclimation genes (35).

TABLE 4. pH 2.5-dependent HP0244 regulon

Functional category	ORF	Wild-type strain/ΔHP0244 strain ratio[a]			Description
		pH 7.4	pH 4.5	pH 2.5	
Acid acclimation	HP0069[c,f]	1.33	1.06	2.47	Urease accessory protein (*ureF*)
	HP1399[b,f]	1.22	1.47	2.85	Arginase (*rocF*)
	HP0723[b]	1.77	1.49	2.28	L-Asparaginase II (*ansB*)
Energy metabolism	HP1133	1.48	1.30	2.49	ATP synthase F1, subunit gamma (*atpG*)
	HP1212	1.66	1.63	3.08	ATP synthase F0, subunit c (*atpE*)
	HP0588[b,e]	1.65	1.96	2.92	Ferrodoxin-like protein
	HP0591[b]	1.63	1.74	2.30	Ferredoxin oxidoreductase, gamma subunit
	HP1099[b]	1.69	1.70	3.48	2-Keto-3-deoxy-6-phosphogluconate aldolase (*eda*)
	HP1193[b]	1.61	1.43	3.43	Aldo-keto reductase, putative
	HP1161	1.79	1.79	2.77	Flavodoxin (*fldA*)
	HP1540[e]	1.68	1.56	2.55	Ubiquinol cytochrome *c* oxidoreductase (*fbcF*)
	HP0265[e]	1.72	1.34	2.57	Cytochrome *c* biogenesis protein (*ccdA*)
	HP0779	1.96	1.88	3.80	Aconitase B (*acnB*)
Oxidative stress	HP1563[f]	1.47	1.88	3.32	Alkyl hydroperoxide reductase (*ahpC*)
	HP0825[e,f]	1.74	1.94	2.82	Thioredoxin reductase (*trxR*)
	HP0874[b,d]	1.33	1.46	3.26	Catalase (KatA)-associated protein (*kapA*)
Amino acid biosynthesis	HP0380[b,c,f]	1.61	1.51	2.37	Glutamate dehydrogenase (*gdhA*)
	HP0330	1.63	1.34	2.31	Ketol-acid reductoisomerase (*ilvC*)
	HP1038	1.69	1.53	2.67	3-Dehydroquinase type II (*aroQ*)
	HP1210	1.36	0.93	2.91	Serine acetyltransferase (*cysE*)
Biosynthesis of cofactors	HP1058	1.63	1.17	2.55	3-Methyl-2-oxob hydroxymethyltransferase (*panB*)
	HP0306[b,c,f]	1.34	1.64	2.46	Glutamate-1-semialdehyde-2,1-aminomutase (*hemL*)
Cell envelope	HP0025[b]	1.23	1.56	2.56	Outer membrane protein (*omp2*)
	HP0706	1.72	1.32	2.49	Outer membrane protein (*omp15*)
	HP0912	1.55	1.58	2.26	Outer membrane protein (*omp20*)
	HP0913	1.31	1.33	2.05	Outer membrane protein (*omp21*)
	HP1157	1.81	1.71	2.46	Outer membrane protein (*omp26*)
	HP1564[f]	1.54	1.06	3.43	Outer membrane protein
	HP0788	1.85	1.36	3.00	Predicted outer membrane protein (*hofF*)
	HP1167	1.43	1.37	2.42	Predicted outer membrane protein (*hofH*)
	HP0196[e]	1.43	1.38	2.16	UDP-3-*O*-glucosamine *N*-acyltransferase (*lpxD*)
	HP1052	1.76	1.22	2.51	UDP-3-*O*-acyl *N*-acetylglucosamine deacetylase (*envA*)
	HP1375	1.43	1.34	2.51	UDP-*N*-acetylglucosamine acyltransferase (*lpxA*)
	HP0160	1.53	1.21	2.36	Conserved hypothetical secreted protein
	HP0003	1.73	1.77	2.56	Octulosonic acid 8-phosphate synthetase (*kdsA*)
	HP0410	1.50	1.20	2.33	Neuraminyllactose-binding hemagglutinin (*hpaA*)
Cellular processes	HP0978	1.29	1.31	2.43	Cell division protein (*ftsA*)
	HP1069[e]	1.69	1.84	3.50	Cell division protein (*ftsH*)
	HP0979	1.71	1.24	2.58	Cell divison protein (*ftsZ*)
	HP1067[b]	1.61	1.27	2.29	Chemotaxis protein (*cheY*)
	HP0243[b,f]	1.32	1.46	3.73	Neutrophil activating protein (*napA*)
	HP0232[e]	1.73	1.39	2.50	Secreted protein involved in flagellar motility
	HP1192[b,d,f]	1.61	1.28	2.59	Secreted protein involved in flagellar motility
	HP0485[b]	1.85	1.58	3.26	Catalase-like protein
	HP1043	1.47	1.51	2.45	Response regulator
	HP1104[b,e,f]	1.66	1.74	3.20	Cinnamyl-alcohol dehydrogenase ELI3-2 (*cad*)
	HP0569	1.80	1.79	3.01	GTP-binding protein (*gtp1*)
	HP0480[e]	1.63	1.30	2.39	GTP-binding protein, fusA-homolog (*yihK*)
Purines, pyrimidines, and nucleotides	HP0618	1.73	1.63	2.41	Adenylate kinase (*adk*)
	HP0045	1.43	1.49	2.37	Nodulation protein (*nolK*)
	HP0409	1.43	1.60	2.40	GMP synthase (*guaA*)
Central intermediate metabolism	HP0020[b]	1.45	1.67	2.95	Carboxynorspermidine decarboxylase (*nspC*)
	HP0620	1.51	1.26	2.43	Inorganic pyrophosphatase (*ppa*)
Fatty acid and phospholipid metabolism	HP0202	1.40	1.46	2.68	Beta-ketoacyl-acyl carrier protein synthase III (*fabH*)
	HP0559	1.53	1.20	2.29	Acyl carrier protein (*acpP*)
	HP0370[e]	1.30	1.37	2.61	Biotin carboxylase (*accC*)

Continued on following page

TABLE 4—*Continued*

Functional category	ORF	Wild-type strain/ΔHP0244 strain ratio[a]			Description
		pH 7.4	pH 4.5	pH 2.5	
	HP0195	1.75	1.34	2.26	Enoyl-(acyl carrier protein) reductase (NADH) (*fabI*)
	HP0215	1.58	1.40	2.25	CDP-diglyceride synthetase (*cdsA*)
Protein fate and translation	HP0210	1.75	1.77	2.44	Chaperone and heat shock protein C62.5 (*htpG*)
	HP0110[b,e]	1.43	0.91	3.07	Cochaperone and heat shock protein (*grpE*)
	HP1202	1.27	1.54	2.44	Ribosomal protein L11 (*rpl11*)
	HP1068	1.71	1.83	3.18	Ribosomal protein L11 methyltransferase (*prmA*)
Transport and binding protein	HP1073	1.43	1.50	2.46	Copper ion binding protein (*copP*)
	HP1562[e]	1.60	1.40	2.92	Iron(III) ABC transporter (*ceuE*)
	HP0302[b]	1.58	1.72	2.61	Dipeptide ABC transporter (*dppF*)
	HP1169	0.84	0.90	2.08	Glutamine ABC transporter, permease protein (*glnP*)
Hypothetical	HP1037[e]	1.69	1.95	3.06	Conserved hypothetical protein
	HP0920	1.80	1.81	2.30	Conserved hypothetical integral membrane protein
	HP0151	1.58	1.17	2.45	Conserved hypothetical membrane protein
	HP1285	1.68	1.15	2.46	Conserved hypothetical secreted protein
	HP0318[b,c]	1.57	1.62	2.36	Conserved hypothetical protein
	HP1459	1.51	1.45	2.88	Conserved hypothetical protein
	HP0310[b]	1.87	1.57	3.84	Conserved hypothetical protein
Unknown	HP0113	0.81	0.90	2.71	*H. pylori* predicted coding region HP0113
	HP0218[b]	1.65	1.57	2.74	*H. pylori* predicted coding region HP0218
	HP0231	1.67	1.83	2.54	*H. pylori* predicted coding region HP0231
	HP0423	1.44	1.33	2.61	*H. pylori* predicted coding region HP0423
	HP0486	1.87	1.32	2.31	*H. pylori* predicted coding region HP0486
	HP0605	1.71	1.64	2.37	*H. pylori* predicted coding region HP0605
	HP0719[c]	1.37	1.16	3.04	*H. pylori* predicted coding region HP0719
	HP0773	1.45	1.37	2.23	*H. pylori* predicted coding region HP0773
	HP0953	1.25	1.36	2.53	*H. pylori* predicted coding region HP0953
	HP0996	1.44	1.43	2.61	*H. pylori* predicted coding region HP0996
	HP1247	1.32	1.40	2.49	*H. pylori* predicted coding region HP1247
	HP1334	1.36	0.75	2.57	*H. pylori* predicted coding region HP1334
	HP1358	1.37	1.64	2.48	*H. pylori* predicted coding region HP1358

[a] Average ratios based on three independent microarray hybridizations with separate RNA preparations for each pH condition.
[b] Acid-responsive gene identified by Wen et al. (50).
[c] Acid-responsive gene identified by Merrell et al. (23).
[d] Acid-responsive gene identified by Bury-Mone et al. (8).
[e] Acid-responsive gene identified by Ang et al. (2).
[f] HP0165 regulon gene identified by Pflock et al. (28).

Several reports have shown that the HP0165-HP0166 (ArsSR) two-component system provides one of the signaling pathways that regulate the expression of most of the acid acclimation genes in response to pH 5.0 or 4.5 (28–30, 48, 49). Consistent with these reports, an acid survival analysis with an HP0165 gene-deficient mutant showed that HP0165 is required for growth of *H. pylori* at pH 5.0 (19). The deduced amino acid sequence of HP0165 (1, 44) shows that it is a membrane-bound sensor histidine kinase with two transmembrane domains and a periplasmic, histidine-rich input domain. Therefore, HP0165 belongs to the largest group of sensors, the periplasmic (or extracellular) sensing histidine kinases, which includes proteins with an extracellular sensory domain framed by at least two transmembrane helices (22). Considering its function in acid-responsive regulation and its domain architecture (i.e., membrane topology, number of transmembrane helices, and sequential arrangement of the sensory domain in its N-terminal input domain), HP0165 functions as a periplasmic pH-sensing histidine kinase. However, this sensor kinase might not be sufficient for mounting an adequately robust response to a highly acidic environment.

The sensor histidine kinase HP0244 and its response regulator HP0703 are required for expression of the σ^{54}-dependent flagellar genes (4, 24, 38). The acid survival analysis performed in this study showed that HP0244 is not required for acid survival at pH 4.5 (Fig. 2). A similar finding was obtained in a separate study performed with *H. pylori* strain J99 at pH 5.0 (19). However, both conditions used in the two studies generated only a mildly acidic pH in the cytoplasm (pH 5.3 to 6.5) as measured by using the pH-sensitive cytoplasmic fluorescent probe BCECF-AM (50).

Our results show that HP0244 is required for acid survival when *H. pylori* cells are exposed to a medium with a pH of 2.5 and with 10 mM urea (Fig. 2), whereas the wild type survives at this pH with 1 mM urea (35). The domain architecture of HP0244 based on its deduced amino acid sequence (1, 44) shows that this protein is soluble and belongs to the second-largest group of sensor kinases, the cytoplasmic-sensing histidine kinases (22).

Like findings for HP0165, it has been demonstrated that an isogenic mutant of *H. pylori* P76 with the HP0244 ORF encoding

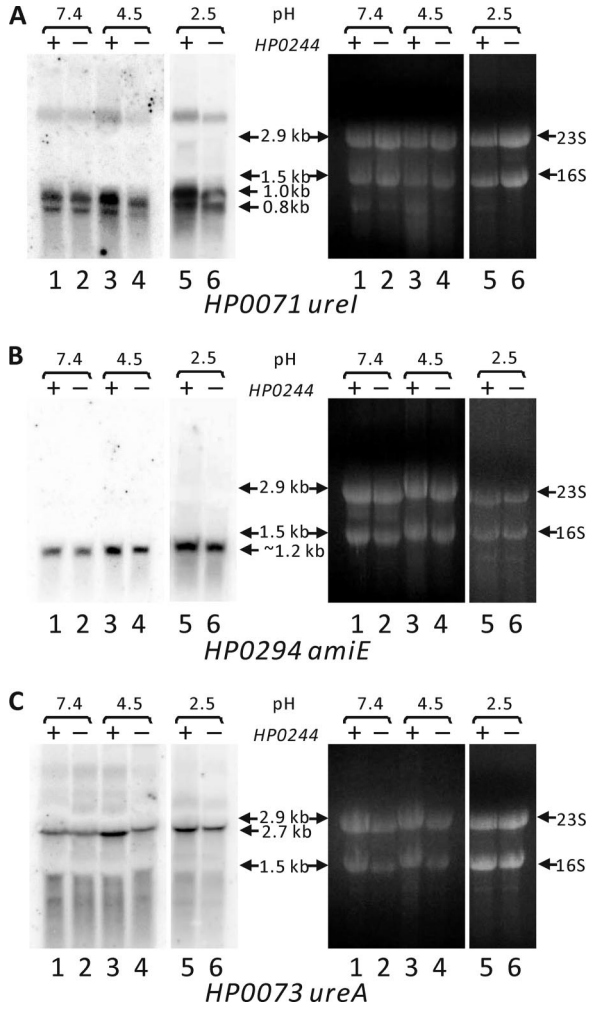

FIG. 4. Low-pH-induced transcription of the HP0071 (*ureI*), HP0294 (*amiE*), and HP0073 (*ureAB*) genes is HP0244 dependent. Total RNAs were extracted from *H. pylori* wild-type strain 26695 (lanes +) and the *flgS* mutant strain 26695/ΔHP0244::km (lanes −) which were exposed to neutral pH (pH 7.4) (lanes 1 and 2), a low pH (pH 4.5 without urea) (lanes 3 and 4), or a lower pH (pH 2.5 with 30 mM urea) (lanes 5 and 6) for 30 min. RNAs were analyzed by Northern blotting with the HP0071 (*ureI*) (A), HP0294 (*amiE*) (B), and HP0073 (*ureA*) (C) gene-specific probes. Ethidium bromide staining of the gel showed that equal amounts of RNA were analyzed. The size standards used were the 16S and 23S rRNA species. The results for pH 2.5 were obtained in experiments separate from the experiments used to obtain the results for pH 7.4 and 4.5.

histidine kinase deleted is unable to colonize the stomachs of BALB/c mice, suggesting that HP0244 has an essential role in the regulation of important virulence properties of *H. pylori* (25). However, HP0244 coordinately regulates motility (6, 38) via its cognate response regulator, HP0703 (FlgR), which has been shown to be an essential colonization factor in *H. pylori* (11, 42). This raises the question of whether the degree of motility of *H. pylori* also correlates with the degree of acid survival in vitro. Our results (Fig. 3) demonstrated that, although HP0244 and HP0703 knockout mutant strains showed equivalent losses of motility (Fig. 3B), the nonmotile HP0703 mutant was able to survive acid treatment at pH 2.5 as well as the wild-type strain (Fig. 3A). Thus, the defect in acid survival of the HP0244 mutant in vitro was not due to a loss of motility. However, it is not possible to distinguish between loss of motility and loss of the acid adaptation response as factors responsible for loss of infectivity, since motility is essential for infection (42).

Whole-genome transcriptional profiling is required for comprehensive analysis of bacterial stimulons and regulons and has been used for characterization of *H. pylori* null mutants with mutations in the HP0244 and HP0703 two-component genes (24). In *H. pylori* strains N6 and 88-3887, the HP0703-HP0244 (FlgRS) two-component system, together with σ^{54} RpoN, was shown to regulate transcription of the class 2 flagellar genes encoding the flagellar hook protein (FlgE1), hook-filament adapter proteins (FlgK and FlgL), and the minor flagellin subunit FlaB. In addition, the HP0869 (*hypA*) gene encoding a nickel-binding protein involved in hydrogenase and urease maturation and some other genes encoding proteins with unknown functions have been found to be regulated by the HP0244-HP0703 two-component system at neutral pH (24).

HP0703 is the response regulator responsible for transcriptional regulation of flagellar genes (4, 38). However, our EMSA results (Fig. 5) show that in vitro-phosphorylated HP0703 does not directly regulate the acid-responsive expression of *ureAB* and *ureI*, as expected from the acid survival results showing that the HP0703 gene-deficient mutant was able to survive acid treatment at pH 2.5 (Fig. 3). Therefore, the transcriptional regulation of the acid acclimation genes in response to acidic pH sensed by HP0244 is not a result of direct transcriptional control by HP0703, suggesting that there must be a pathway outside the HP0703 regulon in the cytoplasm which transduces the acid-responsive signal sensed by HP0244.

Acid survival studies (Fig. 2 and 3A) showed that HP0244 is required for acid survival of *H. pylori* at pH 2.5 with 10 mM urea. Further, the transcription levels of several genes at both pH 4.5 and 2.5 were lower in the HP0244 deletion mutant. This suggests that besides its role in regulating flagellar biosynthesis, HP0244 may also play an important role in *H. pylori* by regulating gene expression in response to a decrease in the pH. Thus, HP0244 may have an acid-dependent regulon that responds to low pH and is distinct from the regulon involved in flagellar gene expression.

To identify the pH-dependent HP0244 regulon, we compared the gene expression of the *H. pylori* 26695 wild-type and 26695/ΔHP0244::km mutant strains at pH 7.4 and under acidic pH conditions (pH 4.5 and 2.5) by using microarray analysis. In addition to a pH-independent HP0244 regulon that is constantly regulated by HP0244 under neutral and both acidic pH conditions tested, there is also a two-part pH-dependent HP0244 regulon consisting of 22 genes specifically regulated by HP0244 in response to pH 4.5 (Table 3) and 86 genes that are regulated by HP0244 at pH 2.5 (Table 4). Seven of the 12 acid acclimation group of genes (33, 35) (*ureA*, *ureB*, *ureI*, *ureF*, *amiE*, *rocF*, and *ansB*) potentially involved in pH homeostasis (50) were found to be in the pH-dependent HP0244 regulon (Tables 3 and 4). Six of these acid acclimation genes are also in the HP0165 regulon (28), showing the overlap between the two histidine kinase systems. However, about 82% of the genes in the pH-dependent HP0244 regulon were not present in the HP0165 regulon, eliminating the possibility that the two histidine kinases (HP0244 and HP0165) use only a common signaling pathway of the response regulator HP0166 for transcrip-

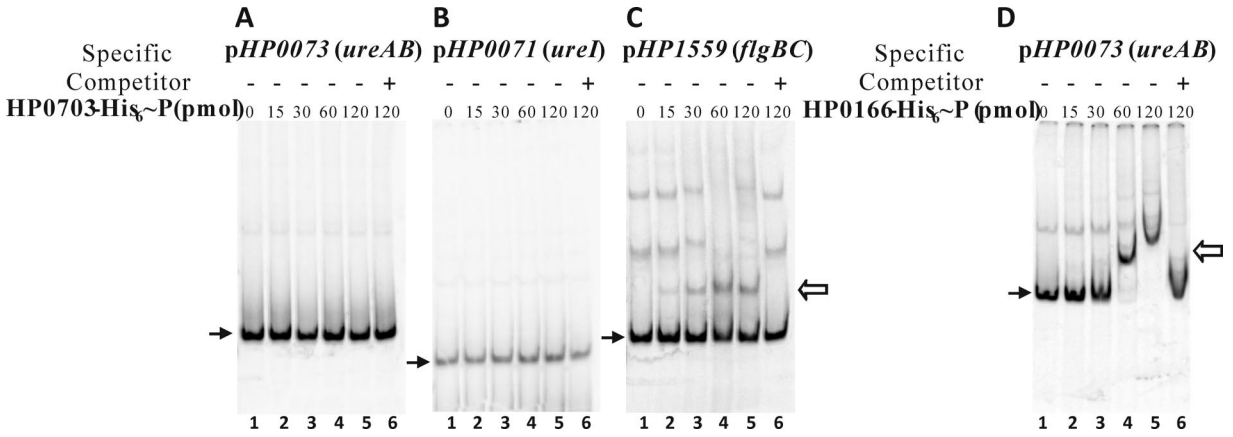

FIG. 5. EMSA with HP0703-His_6 purified from *E. coli* and subsequently phosphorylated in vitro with carbamylphosphate (A, B, and C) and with phosphorylated HP0166-His_6 (D). A 250-bp probe for pHP0073-HP0072 (*ureAB*) (A and D), a 191-bp probe for pHP0071 (*ureI*) (B), and a 206-bp probe for pHP1559-HP1558 (*flgBC*) (C) were used. PCR products for promoter probes were labeled with [γ-^{33}P]ATP. Labeled probes were incubated in the absence of response regulator protein (lane 1), in the presence of different amounts of response regulator protein (15, 30, 60, and 120 pmol) (lanes 2 to 5, respectively), or in the presence of both the protein (120 pmol) and a 50-fold excess of the unlabeled probe as a specific competitor (lane 6). The open and solid arrows indicate the positions of the shifted bands and free probes.

tional regulation. The pH-responsive HP0244 regulon includes not only some acid acclimation genes that may contribute to pH homeostasis but also genes that may reflect a more general stress response induced by an acidic pH. Similarly, the periplasm-sensing kinase HP0165 regulates several pH homeostatic genes, as well as other genes that are expressed as a function of pH changes largely in the periplasm (30).

Soluble cytoplasm-sensing histidine kinases are able to respond to environmental stimuli that have direct access to the cell cytoplasm by transmission (e.g., light for phytochrome photoreceptors) (15, 45) or diffusion (including H_2, H^+, etc.). In *Ralstonia eutropha*, expression of the genes encoding the hydrogenase and of the accessory genes requires activation by a soluble cytoplasm-sensing two-component system, the HoxJ and HoxA proteins (5, 17). Recognition of H_2 is mediated by a signal transduction/sensory complex formed by the histidine kinase HoxJ and a sensory Ni-Fe hydrogenase, HoxBC, which functions as the H_2 sensor (16, 18, 36). In responding to the level of H_2, HoxJ turns on and off its autophosphorylation with the histidine kinase activity, resulting in phosphorylation and dephosphorylation of the cognate response regulator HoxA, which leads to transcriptional regulation of the hydrogenase genes.

It is not clear how changes in pH are recognized by HP0244. A domain architecture analysis of the N-terminal amino acid sequence of HP0244 (excluding the histidine kinase domain in the C terminus) with Simple Modular Architecture Research Tool (http://smart.embl-heidelberg.de/) revealed a PAS domain that starts at position 86 and ends at position 152. PAS domains (initially identified in the Per, Arnt, and Sim proteins) are involved in many signaling proteins, where they are used as signal sensor domains in the cytoplasm (32). PAS domains can sense environmental factors that cross the cell membrane, such as light, redox potential, oxygen, and small ligands, and the overall energy level of a cell by monitoring the proton motive force (40, 52). The latter changes as a function of the pH gradient across the inner membrane. The PAS domain may also play a role in protein-protein interaction as it has a concave front surface similar to that of PAS domains known to interact with other proteins (51). Therefore, pH changes may be recognized by HP0244 by formation of a pH sensory complex with other proteins via the PAS domain of this kinase.

The current study provided evidence that the sensor HP0244 is involved in the acid adaptation response to low medium pH that may result in a significantly acidic cytoplasmic pH. This sensor complements the HP0165 sensor that generates an acid adaptation response to a decrease in the periplasmic pH via HP0166 as the response regulator. Hence, *H. pylori* has at least two two-component histidine kinases that respond to pH changes in both the periplasm and cytoplasm, which results in a pH-responsive network that is likely required for efficient acid adaptation to the varying gastric environment in which this pathogen lives.

ACKNOWLEDGMENTS

This work was supported by U.S. Veterans Administration and NIH grants DK46917, 53462, and 58333 and by a pilot and feasibility grant from The CURE Digestive Diseases Research Center at UCLA.

REFERENCES

1. **Alm, R. A., L. S. Ling, D. T. Moir, B. L. King, E. D. Brown, P. C. Doig, D. R. Smith, B. Noonan, B. C. Guild, B. L. deJonge, G. Carmel, P. J. Tummino, A. Caruso, M. Uria-Nickelsen, D. M. Mills, C. Ives, R. Gibson, D. Merberg, S. D. Mills, Q. Jiang, D. E. Taylor, G. F. Vovis, and T. J. Trust.** 1999. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature **397:**176–180.
2. **Ang, S., C. Z. Lee, K. Peck, M. Sindici, U. Matrubutham, M. A. Gleeson, and J. T. Wang.** 2001. Acid-induced gene expression in *Helicobacter pylori*: study in genomic scale by microarray. Infect. Immun. **69:**1679–1686.
3. **Baker, L. M., A. Raudonikiene, P. S. Hoffman, and L. B. Poole.** 2001. Essential thioredoxin-dependent peroxiredoxin system from *Helicobacter pylori*: genetic and kinetic characterization. J. Bacteriol. **183:**1961–1973.
4. **Beier, D., and R. Frank.** 2000. Molecular characterization of two-component systems of *Helicobacter pylori*. J. Bacteriol. **182:**2068–2076.
5. **Bernhard, M., T. Buhrke, B. Bleijlevens, A. L. De Lacey, V. M. Fernandez, S. P. Albracht, and B. Friedrich.** 2001. The H2 sensor of *Ralstonia eutropha*. Biochemical characteristics, spectroscopic properties, and its interaction with a histidine protein kinase. J. Biol. Chem. **276:**15592–15597.
6. **Brahmachary, P., M. G. Dashti, J. W. Olson, and T. R. Hoover.** 2004. *Helicobacter pylori* FlgR is an enhancer-independent activator of σ^{54}-RNA polymerase holoenzyme. J. Bacteriol. **186:**4535–4542.
7. **Bury-Mone, S., G. L. Mendz, G. E. Ball, M. Thibonnier, K. Stingl, C. Ecobichon, P. Ave, M. Huerre, A. Labigne, J. M. Thiberge, and H. De Reuse.** 2008. Roles of α and β carbonic anhydrases of *Helicobacter pylori* in the urease-dependent response to acidity and in colonization of the murine gastric mucosa. Infect. Immun. **76:**497–509.

8. **Bury-Mone, S., J. M. Thiberge, M. Contreras, A. Maitournam, A. Labigne, and H. De Reuse.** 2004. Responsiveness to acidity via metal ion regulators mediates virulence in the gastric pathogen *Helicobacter pylori*. Mol. Microbiol. **53:**623–638.
9. **Church, G. M., and W. Gilbert.** 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA **81:**1991–1995.
10. **Cooksley, C., P. J. Jenks, A. Green, A. Cockayne, R. P. Logan, and K. R. Hardie.** 2003. NapA protects *Helicobacter pylori* from oxidative stress damage, and its production is influenced by the ferric uptake regulator. J. Med. Microbiol. **52:**461–469.
11. **Eaton, K. A., S. Suerbaum, C. Josenhans, and S. Krakowka.** 1996. Colonization of gnotobiotic piglets by *Helicobacter pylori* deficient in two flagellin genes. Infect. Immun. **64:**2445–2448.
12. **Foster, J. W.** 1999. When protons attack: microbial strategies of acid adaptation. Curr. Opin. Microbiol. **2:**170–174.
13. **Harris, A. G., J. E. Wilson, S. J. Danon, M. F. Dixon, K. Donegan, and S. L. Hazell.** 2003. Catalase (KatA) and KatA-associated protein (KapA) are essential to persistent colonization in the *Helicobacter pylori* SS1 mouse model. Microbiology **149:**665–672.
14. **Hoch, J. A.** 2000. Two-component and phosphorelay signal transduction. Curr. Opin. Microbiol. **3:**165–170.
15. **Karniol, B., J. R. Wagner, J. M. Walker, and R. D. Vierstra.** 2005. Phylogenetic analysis of the phytochrome superfamily reveals distinct microbial subfamilies of photoreceptors. Biochem. J. **392:**103–116.
16. **Kleihues, L., O. Lenz, M. Bernhard, T. Buhrke, and B. Friedrich.** 2000. The H_2 sensor of *Ralstonia eutropha* is a member of the subclass of regulatory [NiFe] hydrogenases. J. Bacteriol. **182:**2716–2724.
17. **Lenz, O., M. Bernhard, T. Buhrke, E. Schwartz, and B. Friedrich.** 2002. The hydrogen-sensing apparatus in *Ralstonia eutropha*. J. Mol. Microbiol. Biotechnol. **4:**255–262.
18. **Lenz, O., and B. Friedrich.** 1998. A novel multicomponent regulatory system mediates H_2 sensing in *Alcaligenes eutrophus*. Proc. Natl. Acad. Sci. USA **95:**12474–12479.
19. **Loh, J. T., and T. L. Cover.** 2006. Requirement of histidine kinases HP0165 and HP1364 for acid resistance in *Helicobacter pylori*. Infect. Immun. **74:** 3052–3059.
20. **Marcus, E. A., A. P. Moshfegh, G. Sachs, and D. R. Scott.** 2005. The periplasmic α-carbonic anhydrase activity of *Helicobacter pylori* is essential for acid acclimation. J. Bacteriol. **187:**729–738.
21. **Marshall, B. J., and J. R. Warren.** 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **i:**1311–1315.
22. **Mascher, T., J. D. Helmann, and G. Unden.** 2006. Stimulus perception in bacterial signal-transducing histidine kinases. Microbiol. Mol. Biol. Rev. **70:**910–938.
23. **Merrell, D. S., M. L. Goodrich, G. Otto, L. S. Tompkins, and S. Falkow.** 2003. pH-regulated gene expression of the gastric pathogen *Helicobacter pylori*. Infect. Immun. **71:**3529–3539.
24. **Niehus, E., H. Gressmann, F. Ye, R. Schlapbach, M. Dehio, C. Dehio, A. Stack, T. F. Meyer, S. Suerbaum, and C. Josenhans.** 2004. Genome-wide analysis of transcriptional hierarchy and feedback regulation in the flagellar system of *Helicobacter pylori*. Mol. Microbiol. **52:**947–961.
25. **Panthel, K., P. Dietz, R. Haas, and D. Beier.** 2003. Two-component systems of *Helicobacter pylori* contribute to virulence in a mouse infection model. Infect. Immun. **71:**5381–5385.
26. **Parkinson, J. S., and E. C. Kofoid.** 1992. Communication modules in bacterial signaling proteins. Annu. Rev. Genet. **26:**71–112.
27. **Pflock, M., M. Bathon, J. Schar, S. Muller, H. Mollenkopf, T. F. Meyer, and D. Beier.** 2007. The orphan response regulator HP1021 of *Helicobacter pylori* regulates transcription of a gene cluster presumably involved in acetone metabolism. J. Bacteriol. **189:**2339–2349.
28. **Pflock, M., N. Finsterer, B. Joseph, H. Mollenkopf, T. F. Meyer, and D. Beier.** 2006. Characterization of the ArsRS regulon of *Helicobacter pylori*, involved in acid adaptation. J. Bacteriol. **188:**3449–3462.
29. **Pflock, M., S. Kennard, I. Delany, V. Scarlato, and D. Beier.** 2005. Acid-induced activation of the urease promoters is mediated directly by the ArsRS two-component system of *Helicobacter pylori*. Infect. Immun. **73:**6437–6445.
30. **Pflock, M., S. Kennard, N. Finsterer, and D. Beier.** 2006. Acid-responsive gene regulation in the human pathogen *Helicobacter pylori*. J. Biotechnol. **126:**52–60.
31. **Pflock, M., S. Muller, and D. Beier.** 2007. The CrdRS (HP1365-HP1364) two-component system is not involved in pH-responsive gene regulation in the *Helicobacter pylori* strains 26695 and G27. Curr. Microbiol. **54:**320–324.
32. **Ponting, C. P., and L. Aravind.** 1997. PAS: a multifunctional domain family comes to light. Curr. Biol. **7:**R674–R677.
33. **Sachs, G., J. A. Kraut, Y. Wen, J. Feng, and D. R. Scott.** 2006. Urea transport in bacteria: acid acclimation by gastric *Helicobacter* spp. J. Membr. Biol. **212:**71–82.
34. **Sachs, G., D. L. Weeks, K. Melchers, and D. R. Scott.** 2003. The gastric biology of *Helicobacter pylori*. Annu. Rev. Physiol. **65:**349–369.
35. **Sachs, G., D. L. Weeks, Y. Wen, E. A. Marcus, D. R. Scott, and K. Melchers.** 2005. Acid acclimation by *Helicobacter pylori*. Physiology (Bethesda) **20:**429–438.
36. **Schwartz, E., U. Gerischer, and B. Friedrich.** 1998. Transcriptional regulation of *Alcaligenes eutrophus* hydrogenase genes. J. Bacteriol. **180:**3197–3204.
37. **Scott, D. R., E. A. Marcus, Y. Wen, J. Oh, and G. Sachs.** 2007. Gene expression in vivo shows that *Helicobacter pylori* colonizes an acidic niche on the gastric surface. Proc. Natl. Acad. Sci. USA **104:**7235–7240.
38. **Spohn, G., and V. Scarlato.** 1999. Motility of *Helicobacter pylori* is coordinately regulated by the transcriptional activator FlgR, an NtrC homolog. J. Bacteriol. **181:**593–599.
39. **Stock, A. M., V. L. Robinson, and P. N. Goudreau.** 2000. Two-component signal transduction. Annu. Rev. Biochem. **69:**183–215.
40. **Taylor, B. L., and I. B. Zhulin.** 1999. PAS domains: internal sensors of oxygen, redox potential, and light. Microbiol. Mol. Biol. Rev. **63:**479–506.
41. **Taylor, L. A., and R. E. Rose.** 1988. A correction in the nucleotide sequence of the Tn903 kanamycin resistance determinant in pUC4K. Nucleic Acids Res. **16:**358.
42. **Terry, K., S. M. Williams, L. Connolly, and K. M. Ottemann.** 2005. Chemotaxis plays multiple roles during *Helicobacter pylori* animal infection. Infect. Immun. **73:**803–811.
43. **Teyssen, S., S. T. Chari, J. Scheid, and M. V. Singer.** 1995. Effect of repeated boluses of intravenous omeprazole and primed infusions of ranitidine on 24-hour intragastric pH in healthy human subjects. Dig. Dis. Sci. **40:**247–255.
44. **Tomb, J. F., O. White, A. R. Kerlavage, R. A. Clayton, G. G. Sutton, R. D. Fleischmann, K. A. Ketchum, H. P. Klenk, S. Gill, B. A. Dougherty, K. Nelson, J. Quackenbush, L. Zhou, E. F. Kirkness, S. Peterson, B. Loftus, D. Richardson, R. Dodson, H. G. Khalak, A. Glodek, K. McKenney, L. M. Fitzegerald, N. Lee, M. D. Adams, J. C. Venter, et al.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:**539–547.
45. **Vierstra, R. D., and S. J. Davis.** 2000. Bacteriophytochromes: new tools for understanding phytochrome signal transduction. Semin. Cell Dev. Biol. **11:** 511–521.
46. **Waidner, B., K. Melchers, F. N. Stahler, M. Kist, and S. Bereswill.** 2005. The *Helicobacter pylori* CrdRS two-component regulation system (HP1364/HP1365) is required for copper-mediated induction of the copper resistance determinant CrdA. J. Bacteriol. **187:**4683–4688.
47. **Wang, Y., and D. E. Taylor.** 1990. Chloramphenicol resistance in *Campylobacter coli*: nucleotide sequence, expression, and cloning vector construction. Gene **94:**23–28.
48. **Wen, Y., J. Feng, D. R. Scott, E. A. Marcus, and G. Sachs.** 2007. HP0165-HP0166 two-component system (ArsRS) regulates the acid-induced expression of HP1186 α-carbonic anhydrase in *Helicobacter pylori* by activating the pH-dependent promoter. J. Bacteriol. **189:**2426–2434.
49. **Wen, Y., J. Feng, D. R. Scott, E. A. Marcus, and G. Sachs.** 2006. Involvement of the HP0165-HP0166 two-component system in expression of some acidic-pH-upregulated genes of *Helicobacter pylori*. J. Bacteriol. **188:**1750–1761.
50. **Wen, Y., E. A. Marcus, U. Matrubutham, M. A. Gleeson, D. R. Scott, and G. Sachs.** 2003. Acid-adaptive genes of *Helicobacter pylori*. Infect. Immun. **71:** 5921–5939.
51. **Yildiz, O., M. Doi, I. Yujnovsky, L. Cardone, A. Berndt, S. Hennig, S. Schulze, C. Urbanke, P. Sassone-Corsi, and E. Wolf.** 2005. Crystal structure and interactions of the PAS repeat region of the *Drosophila* clock protein PERIOD. Mol. Cell **17:**69–82.
52. **Zhulin, I. B., B. L. Taylor, and R. Dixon.** 1997. PAS domain S-boxes in Archaea, Bacteria and sensors for oxygen and redox. Trends Biochem. Sci. **22:**331–333.