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Helicobacter pylori ABC Transporter: Effect of Allelic Exchange Mutagenesis on Urease Activity

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Helicobacter pylori **urease requires nickel ions in the enzyme active site for catalytic activity. Nickel ions must, therefore, be actively acquired by the bacterium. NixA (high-affinity nickel transport protein)-deficient mutants of *H. pylori* retain significant urease activity, suggesting the presence of alternate nickel transporters. Analysis of the nucleotide sequence of the *H. pylori* genome revealed a homolog of NikD, a component of an ATP-dependent nickel transport system in *Escherichia coli*. Based on this sequence, a 378-bp DNA fragment was PCR amplified from *H. pylori* genomic DNA and used as a probe to identify an *H. pylori* λZAPII genomic library clone that carried these sequences. Four open reading frames of 621, 273, 984, and 642 bp (*abcABCD*) were revealed by sequencing and predicted polypeptides of 22.7, 9.9, 36.6, and 22.8 kDa, respectively. The 36.6-kDa polypeptide (AbcC) has significant homology (56% amino acid sequence identity) to an *E. coli* ATP-binding protein component of an ABC transport system, while none of the other putative proteins are significantly homologous to polypeptides in the available databases. To determine the possible contribution of these genes to urease activity, *abcC* and *abcD* were each insertionally inactivated with a kanamycin resistance (*aphA*) cassette and allelic exchange mutants of each gene were constructed in *H. pylori* UMAB41. Mutation of *abcD* resulted in an 88% decrease in urease activity to 27 ± 31 μmol of NH_3/min/mg of protein ($P < 0.0001$), and a double mutant of *nixA* and *abcC* resulted in the near abolishment of urease activity (1.1 ± 1.4 μmol of NH_3/min/mg of protein in the double mutant versus 228 ± 92 μmol of NH_3/min/mg of protein in the parent [$P < 0.0001$]). Synthesis of urease apoenzyme, however, was unaffected by mutations in any of the *abc* genes. We conclude that the *abc* gene cluster, in addition to *nixA*, is involved in production of a catalytically active urease.**

Peptic ulcer disease is a widespread ailment that may affect as much as 4% of women and 10% of men over their lifetimes (48). *Helicobacter pylori*, a gram-negative, microaerophilic, spiral-shaped bacterium, has been established as an important cause of gastritis (7, 58). Infection with this organism is strongly associated with the development of gastric and duodenal ulcers (10, 33, 36); chronic infection has also been correlated with the progression to gastric carcinoma (44). The mechanisms by which *H. pylori* colonizes the acidic gastric mucosa and causes these diseases are not yet fully understood; however, one cellular protein, urease, is required for colonization (14) and most likely contributes directly to peptic ulcer disease (for a review, see reference 42). Urease catalyzes the hydrolysis of urea to generate carbon dioxide and ammonia. The ammonia produced by this reaction is hypothesized to allow *H. pylori* to survive in the gastric mucosa by neutralizing gastric acid around the bacterium and raising the local pH (14, 37, 40, 54). The contribution of urease to virulence, however, appears to go well beyond simple protection from acid (15, 51).

Urease is a multimeric, 550-kDa, cytosolic metalloenzyme that requires the divalent cation Ni^{2+} for catalytic activity (13, 24, 40). The absolute requirement of nickel for urease to be active has necessitated an elaborate mechanism for acquisition and incorporation of the ion. Accessory genes within the urease gene cluster, in addition to structural subunit genes *ureA* and *ureB*, play a role in the incorporation of Ni^{2+} into the active site of newly synthesized apoenzyme (28, 42). Soluble proteins encoded by these genes handle Ni^{2+} that has accumulated in the cytosol in a process that may require ATP. Other *H. pylori* proteins such as NixA, a high-affinity Ni^{2+} transporter, play a role in this process by importing Ni^{2+} ions into the cell (41). An isogenic *nixA* mutant, however, still retains about half of its urease activity relative to the wild-type parent strain, suggesting that additional proteins may be responsible for transporting nickel into the cell (5).

Inspection of DNA sequence from the *H. pylori* genome resulted in the discovery of a short sequence predicting a polypeptide which is homologous to a protein component of an *Escherichia coli* ATP-binding cassette (ABC) family of bacterial transporters. This *E. coli* homolog, *nikD*, encodes a nickel transport system component (43). Our work has centered on characterizing the *nikD* homolog in *H. pylori* and determining its contribution to synthesis of a catalytically active urease.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *H. pylori* UMAB41 was originally obtained from a patient with suspected gastritis at the time of endoscopic examination. Biopsied tissue was used to inoculate brucella agar plates (Difco Laboratories) containing 10% sheep blood and Skirrow's supplement (50) and was cultured for 72 h at 37°C under microaerobic conditions (atmosphere generated by a CampyPak [Becton Dickinson]). *H. pylori* was identified based upon spiral morphology, gram-negative staining, and positive catalase, oxidase, and urease activities. Pure cultures were stored in Luria broth supplemented with 15% (vol/vol) glycerol at −70°C. Mutant strains containing antibiotic resistance cassettes were cultured identically to UMAB41 except for the addition of kanamycin (30 μg/ml) and chloramphenicol (20 μg/ml) to the medium as appropriate.

E. coli DH5α was used in cloning experiments (2). Antibiotic-resistant clones were maintained on Luria agar supplemented with either ampicillin (50 μg/ml), kanamycin (30 μg/ml), chloramphenicol (20 μg/ml), or, where appropriate, a combination of these antibiotics. For insertional inactivation experiments in which loss of *lacZ* expression was sought, Luria agar plates were spread with 20 μl of a 120-mg/ml isopropyl-β-D-thiogalactopyranoside (IPTG) concentration for an approximate concentration of 120 μg/ml and 40 μl of a 20-mg/ml concentration of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) for an approximate concentration of 40 μg/ml.

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TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Characteristic(s)	Source or reference
E. coli		
DH5α	*supE44 ΔlacU169 ϕ80 lacZΔM15/hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	2
HB101	*supE44 hsdS20*(r_B^- m_B^-) *recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*	2
H. pylori		
UMAB41	Clinical isolate cultured from gastric biopsy	40
HP-ABC3	*abcC* allelic exchange mutant of UMAB41 (Kan^r)	This work
HP-ABC4	*abcD* allelic exchange mutant of UMAB41 (Kan^r)	This work
HP-NIXA	*nixA* allelic exchange mutant of UMAB41 (Cm^r)	This work
HP-ABC3N	*abcC-nixA* allelic exchange mutant of UMAB41 (Kan^r Cm^r)	This work
Plasmids		
pCR-Script SK(+)	Cloning vector (Ap^r)	Stratagene
pHP1	Contains *aphA* kanamycin resistance cassette on 1.4-kb *Eco*RI fragment (Ap^r Kan^r)	H. Kleanthous; 53
pSKCAT4	Contains *cat* chloramphenicol resistance cassette on 1.5-kb *Sma*I fragment (Ap^r Cm^r)	D. Taylor; 57
pNIK17	pCR-Script SK(+) with the insertion of 378-bp *nikD* homolog PCR product at a *Srf*I site (Ap^r)	This work
pZAP9-1F	λZAPII genomic library clone containing *abc* sequences (Ap^r)	This work
p30-10A	pHC79-based cosmid genomic library clone containing *abc* sequences (Ap^r)	This work
pABC103	Subclone of pZAP9-1F; pCR-Script SK(+) containing *abc* sequences on a 2.4-kb PCR amplified fragment (Ap^r)	This work
pABC203	pABC103 with the insertion of a Kan^r cassette at the *Sty*I site within *abcC* (Ap^r Kan^r)	This work
pABC204	pABC103 with the insertion of a Kan^r cassette at the *Nhe*I site within *abcD* (Ap^r Kan^r)	This work
pUEF202	pBluescript vector with a ~2.4-kb *Hin*dIII fragment containing *nixA*	41
pUEF411	pUEF202 with the insertion of a Cm^r cassette at the *Pfl*MI site within *nixA*	This work

The host strain for the pHC79-based cosmid genomic library, *E. coli* HB101, was cultured similarly to DH5α and maintained on Luria agar supplemented with ampicillin (50 μg/ml) (2). For long-term storage, pure cultures were stored in Luria broth supplemented with 15% (vol/vol) glycerol at −70°C.

Table 1 lists all bacterial strains used in this study.

Plasmids. Plasmid pCR-Script SK(+) (Stratagene) is based on the Bluescript II SK(+) phagemid and contains a *Srf*I restriction endonuclease site which was used for cloning as described by the manufacturer. Plasmid pHP1, generously provided by H. Kleanthous (Oravax), contains a 1.4-kb kanamycin resistance (*aphA*) cassette derived from *Streptococcus faecalis* (53). The *aphA* cassette carries its own promoter and can be removed from pHP1 by digestion with *Eco*RI. Plasmid pSKCAT4, a gift from D. Taylor (University of Alberta, Edmonton, Alberta, Canada) contains a 621-bp chloramphenicol acetyltransferase gene (*cat*) originally derived from *Campylobacter coli* (57). The *cat* gene was isolated from the pBluescriptSK vector on a 1.5-kb *Eco*RI fragment. Plasmid pUEF202, which contains the *nixA* gene, has been described previously (41). New plasmid constructs and relevant *H. pylori* genomic library plasmid isolates are described in Results; all plasmids are listed in Table 1.

TABLE 2. Oligonucleotides used for PCR analysis

Primer pair no.	Sequence (5′-3′)	Target	Conditions	Expected product	Source or reference
1	GTGGTTAAAGAATTGTGC CTTTAATTTTTCCACTTG	*nikD* homolog	Anneal 51°C, 2 min; extend 72°C, 2 min; denature 94°C, 1 min	378 bp	This work
2	GGCTAATCCATCAAATAGC TAATCACAACCACCGCATAA	*abc* sequences	Anneal 51°C, 2 min; extend 72°C, 2 min; denature 94°C, 1 min	2.4 kb	This work
3	GAATAGGAGAATGAGATGAA TGACAAAAAATAAAAACAAA	*ureA* and *ureB*	Anneal 51°C, 2 min; extend 72°C, 2 min; denature 94°C, 1 min	2.5 kb	11
4	ATTCCCATGGATTTATTAAAAAT ATTCAAAAAATGC CTGTAGATCTTAGGCTTTGTAAG GTGTTTAGAAG	Region of *nixA*	Anneal 51°C, 2 min; extend 72°C, 2 min; denature 94°C, 1 min	517 bp, 2.0 kb[a]	This work
5	ATTGTGCGATACCTGAAG TGTTGATGGAAGTGTCTA	*abcC*	Anneal 46°C, 2.25 min; extend 72°C, 2 min; denature 94°C, 1 min	1.2 kb, 2.6 kb[a]	This work
6	AATGGGGTCAATCTGTTA ATCCTAATGGCTAAATCC	*abcD*	Anneal 48°C, 2 min; extend 72°C, 2 min; denature 94°C, 1 min	1.3 kb, 2.7 kb[a]	This work

[a] Expected size including antibiotic resistance cassette insertion.

PCR. All oligonucleotide primers and thermal cycle parameters used for PCR analysis are listed in Table 2. Primer pair 1 was used to amplify the 378-bp fragment of the *nikD* homolog (used in colony blot hybridization) from *H. pylori* chromosomal DNA. Primer pair 2 was used to amplify the 2,468-bp *abc* sequence for subcloning. Oligonucleotide primers based on the published DNA sequence of *H. pylori ureA* and *ureB* (primer pair 3) were used to amplify a region of 2,544 bp from genomic DNA (*ureA* and *ureB*) for positive identification of *H. pylori* (11) (GenBank accession no. X17079). To identify *nixA* clones containing the chloramphenicol resistance cassette, primer pair 4 was used to amplify a 517-bp product. The 1,273-bp fragment used for identification of *abcC* clones containing the kanamycin resistance cassette insertion and for Southern hybridizations was amplified with primer pair 5. To identify *abcD* clones containing the kanamycin resistance cassette insertion and for Southern hybridizations, oligonucleotide primer pair 6 was used to amplify a 1,323-bp fragment. All PCRs were run for 30 cycles with *Pfu* DNA polymerase (Stratagene). All oligonucleotide primers were synthesized by the phosphoramidite method on an Applied Biosystems automated DNA synthesizer (model 380B).

DNA isolation. Plasmid DNA was isolated from bacterial cells by either the alkaline-lysis method (6) or a modified alkaline-lysis–chromatography procedure using DNA purification columns (Qiagen) as directed by the manufacturer.

Chromosomal DNA was isolated from *H. pylori* by lysis with sodium dodecyl sulfate (SDS), treatment with proteinase K, and extraction with phenol, chloroform, and isoamyl alcohol (in place of ether) by this modification of the method of Marmur (35).

Recombinant DNA methods. The construction of plasmids pNIK17 and pABC103 was carried out with the Stratagene pCR-Script cloning kit as described in the manufacturer's directions (Stratagene). For construction of other plasmids, recombinant DNA techniques, including restriction endonuclease digestion, ligation, electroporation, and transformation, were carried out by standard protocols (2, 46).

***H. pylori* genomic libraries.** Construction of the λZAPII genomic library and the pooled plasmid library have been described previously (25, 41). Construction of the pHC79-based cosmid library has also been described previously (28).

DNA hybridization. Bacterial colonies grown on nitrocellulose filters were hybridized with a 378-bp DNA probe by the method of Sambrook et al. (46). Oligonucleotide probes were labeled with [α-^{32}P]dATP by random priming as described in the Random-Primed DNA Labeling kit (Boehringer Mannheim). Hybridization was done under stringent conditions (50% formamide, 5× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% SDS, 65°C wash).

Southern blots of PCR products were carried out by standard methods under stringent conditions (0.2× SSC; Qiagen). DNA probes were labeled with horseradish peroxidase and detected with the ECL 3′ oligonucleotide labeling system as described in the manufacturer's directions (Amersham).

Nucleotide sequencing and analysis. Nucleotide sequencing was carried out with a model 373A DNA sequencer (Applied Biosystems), reagents from the Prism Ready-Reaction Dye Deoxy Termination kit (Applied Biosystems), and *Taq* DNA polymerase. The nucleotide sequence of pZAP9-1F was determined with the universal T4 and T7 primers (Stratagene) of pBluescript II SK(+). Additional primers were synthesized on the basis of newly acquired sequence.

Genepro DNA sequence analysis software (Riverside Scientific Enterprises) was used for routine analysis of the nucleotide sequence and predicted polypeptide sequences. The Genetics Computer Group (University of Wisconsin) sequence analysis software package, version 7.3.1, was used to screen the SwissProt database for proteins with sequence similarities.

Urease preparation. *H. pylori* strains grown as described above were harvested from blood agar plates and resuspended in cold PEB buffer (20 mM Na_2HPO_4, 1 mM EDTA, 1 mM β-mercaptoethanol [pH 6.8]). Cells were washed twice in cold PEB buffer and passaged through a French pressure cell at 20,000 lb/in^2. Unbroken cells and insoluble material were removed by centrifugation (10,000 × *g*, 10 min, 4°C). Soluble protein was held on ice until used in Western blotting (immunoblotting) or urease assays.

Urease assay. Rates of urea hydrolysis for soluble protein were measured by the phenol red indicator spectrophotometric assay of Hamilton-Miller and Gargan (23). Quantitative determinations were made as described previously (39). Protein concentrations were determined relative to a bovine serum albumin standard by the bicinchoninic acid method as described in the manufacturer's directions (Pierce).

SDS-PAGE and Western blotting. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described by Sambrook et al. (46). Protein samples were denatured for 5 min at 100°C in 625 mM Tris-HCl (pH 6.8) containing 3% SDS, 10% glycerol, and 5% β-mercaptoethanol. Samples were loaded onto a 12% polyacrylamide (1:29, *N*,*N*′-methylene-bisacrylamide/acrylamide) gel with a 5% stacking gel and electrophoresed at 100 V for 3 h. The gels were stained with 0.2% Coomassie brilliant blue in 50% ethanol–10% acetic acid and destained in 10% ethanol–5% acetic acid.

Western blotting was performed as described by Ausubel et al. (2). Protein (5 μg) was electrophoresed on a 12% polyacrylamide gel as described above and blotted onto an Immobilon P polyvinylidene difluoride membrane (Millipore). All incubations were carried out at room temperature. The blot was blocked in Tween–Tris-buffered saline (TTBS; 100 mM Tris-HCl [pH 7.5], 0.9% NaCl, 0.1% Tween 20) and incubated with rabbit antisera (diluted 1:50,000 in blocking buffer) raised against each of the purified *H. pylori* UreA and UreB urease subunit polypeptides (29). Polyvinylidene difluoride membranes were incubated in antisera for 1 h. After being washed in TTBS, the membranes were incubated in a 1:2,000 dilution of alkaline phosphatase-conjugated goat antirabbit immunoglobulin G (Sigma) for 1 h. Membranes were washed as described above, and the color reaction was developed with Nitro Blue Tetrazolium and 5-bromo-4-chloro-3-indolylphosphate. Protein standards are given below (see legend to Fig. 6).

Nickel ion transport. Nickel ion transport experiments were conducted as described previously (5) with the following modifications: cells were grown to an optical density at 600 nm of 0.5, the specific activity of $^{63}NiCl_2$ was 11 mCi/ml, 0.87 mg of Ni/ml (Amersham) was used, the final $NiCl_2$ concentrations ranged from 50 nM to 1 μM, and samples were taken at 0.25, 0.5, 0.75, 1, 2, 5, and 10 min.

Urease immunoprecipitation. *H. pylori* strains UMAB41 and HP-ABC3 were cultured under microaerobic conditions in Mueller-Hinton broth supplemented with 4% (vol/vol) horse serum (18) and $^{63}NiCl_2$ (specific activity, 11 mCi/ml, 0.87 mg of Ni/ml; Amersham) to concentrations of 1, 2, 5, and 10 μM. Bacteria, harvested by centrifugation (10,000 × *g*, 10 min, 4°C), were washed twice with 10 ml of ice-cold 20 mM sodium phosphate (pH 6.8), resuspended in 1.5 ml of 20 mM sodium phosphate (pH 6.8), and disrupted by a single passage through a French pressure cell at 20,000 lb/in^2. Insoluble material was removed by centrifugation (10,000 × *g*, 10 min, 4°C), and soluble protein was analyzed for urease activity and protein concentration.

Urease was immunoprecipitated with anti-UreB antiserum as described previously (3, 30, 52).

Other enzyme assays. *H. pylori* UMAB41 and HP-ABC3 grown as described above were harvested from blood agar plates and resuspended in cold 10 mM sodium phosphate buffer (pH 6.8). Cells were washed twice in this buffer and passaged through a French pressure cell at 20,000 lb/in^2. Unbroken cells and insoluble material were removed by centrifugation (10,000 × *g*, 10 min, 4°C). Soluble protein was held on ice until used in catalase assays.

Catalase activity was measured as described previously (4). Briefly, soluble protein (3.3 μg) was added to 1 ml of 10 mM sodium phosphate (pH 6.8) containing 11 mM H_2O_2. Catalase activity was measured spectrophotometrically by monitoring the change in absorbance (corresponding to the disappearance of H_2O_2) at 240 nm for 1 min. Assays were conducted at 23°C. Initial rates were calculated based upon a standard absorbance curve of 0 to 11 mM H_2O_2 under the same conditions.

For oxidase assays, whole cells of UMAB41 and HP-ABC3 were harvested from blood agar and washed twice in sodium phosphate buffer as described above. Wild-type and mutant bacteria were resuspended in sodium phosphate buffer to give equal absorbances at 600 nm. A solution (10 μl) containing 1% *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine dihydrochloride (Sigma) was pipetted onto Whatman 3MM Chr chromatography paper, and the bacterial suspension (5 μl) was pipetted onto the middle of each spot. Dilutions of the bacterial suspensions (1:2, 1:4, and 1:8) were also assayed. Oxidase activity was indicated by the appearance of the blue oxidized form of tetramethyl-*p*-phenylenediamine.

Nucleotide sequence accession number. The nucleotide sequence of *abcABCD* has been assigned GenBank accession no. AF010307.

RESULTS

Screening of *H. pylori* genomic libraries. NixA, a high-affinity nickel transport protein of *H. pylori*, has been shown to be a primary provider of intracellular nickel, which is required for synthesis of a catalytically active urease (41). NixA-deficient mutants, however, retain greater than 50% urease activity, suggesting redundant mechanisms by which nickel enters the bacterium (5). DNA sequence with limited homology to *nikD* was identified in an *H. pylori* genome database (C. Clayton and R. Williamson, Glaxo Wellcome) and was generously provided for these studies. *nikD* is one of five genes in an operon that is responsible for providing nickel to hydrogenase, another nickel metalloenzyme in *E. coli*. Our aim was to use these sequence data to determine whether an analogous system was present in *H. pylori*. Nucleotide primers, designed from a DNA sequence obtained from the genome sequence (primer pair 1) (Table 1), were used to PCR amplify a 378-bp fragment of a *nikD* homolog from *H. pylori* UMAB41 chromosomal DNA. The ends of the fragment were rendered blunt by treatment with T4 DNA polymerase and cloned into the *Srf*I restriction site of vector pCR-Script SK(+). The resulting clone, pNIK17, was used as a template for further PCR amplification of the 378-bp fragment. This product was purified from an agarose gel slice, labeled with [α-^{32}P]dATP by random priming, and used to screen both a λZAPII genomic library and a pHC79-based

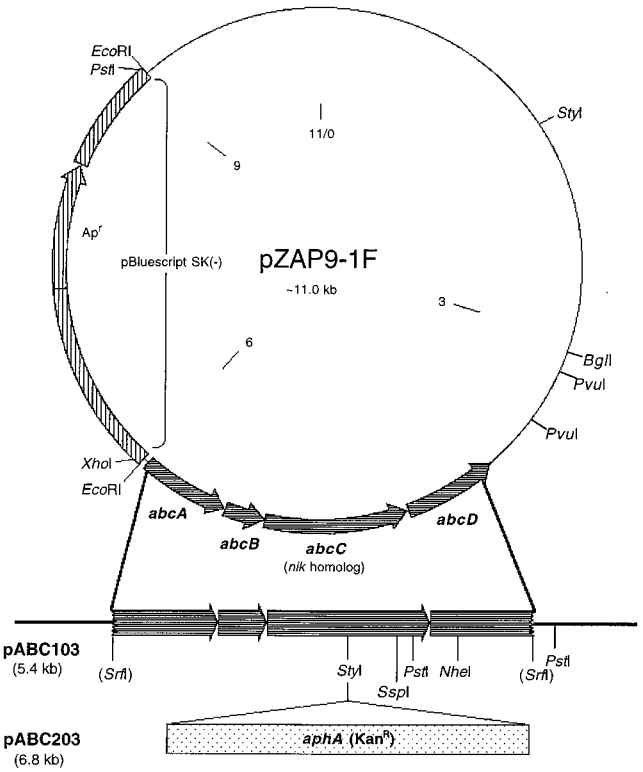

FIG. 1. Restriction map of pZAP9-1F and its subclones. The pBluescript SK(−) region is vertically hatched. Regions representing ABC transporter genes *abcABCD* are horizontally hatched. Relevant restriction sites are shown and confirmed by DNA sequencing. A 2.4-kb fragment amplified by PCR from within *abcABCD* was blunt end ligated into the *Srf*I site of pCR-Script to form plasmid pABC103. *E. coli*, transformed with pABC103, was selected among white colonies on medium containing IPTG, X-Gal, and ampicillin. A 1.4-kb *aphA* (Kan^r) cassette (stippled region) was isolated from plasmid pHP1 by digestion with *Eco*RI, rendered blunt ended with Klenow fragment of *E. coli* DNA polymerase, and ligated into the *Sty*I site of pABC103. Bacterial transformants containing this new plasmid, designated pABC203, were selected for Kan and Amp resistance. pABC203 was electroporated into strain UMAB41 and incubated under microaerobic conditions for 3 days on blood agar containing kanamycin (30 μg/ml). Potential double-crossover mutants (Kan^r colonies) were isolated and analyzed by PCR and Southern blotting. pABC204 (not shown) was constructed identically to pABC203, except that the Kan^r cassette was cloned into the *Nhe*I site of pABC103.

cosmid library of *H. pylori* by colony blot hybridization. One of 1,152 λZAPII gene bank clones tested hybridized strongly with the 378-bp fragment. This clone, pZAP9-1F, contained a genomic insert of 7.5 kb and was used for restriction mapping and sequencing. In addition, 19 of 3,744 pHC79-based cosmid gene bank clones hybridized with the probe. One of these, clone p30-10A, contained a genomic insert of >12 kb and was used in addition to pZAP9-1F for sequencing studies.

***nik* homolog localization.** To assist in choosing primers for sequencing and to ascertain the position of the *nik* homolog relative to the *Eco*RI cloning sites of the genomic insert, a restriction map was determined for pZAP9-1F (Fig. 1). The placement of the *nik* homolog was supported by Southern blots of the digests by use of the 378-bp probe (data not shown). The orientation of the *nik* fragment was determined by digesting pZAP9-1F with *Pst*I and with *Sty*I, which cuts upstream of the *Pst*I site but has no sites in the pBluescript portion of pZAP9-1F.

Nucleotide sequence. Sequencing primers were designed based upon restriction mapping data. The nucleotide sequence of 3,403 bp within the 7.5-kb genomic insert of pZAP9-1F was determined in both directions (Fig. 2). Four open reading frames (ORFs) of 621, 273, 984, and 642 bp were identified and are predicted to be transcribed in the same direction. The largest intergenic region separating any of these genes is 21 bp between *abcB* and *abcC*, suggesting that all four genes are part of a multigene cluster or operon. Potential ribosome binding sites were identified upstream of *abcB*, *abcC*, and *abcD* at bp positions 639 (AGGG), 933 (AGTT), and 1919 (AGGA), respectively. The lack of significant DNA dyad symmetry downstream of *abcD* suggests that transcription is not terminated in a rho-independent manner. The G+C content of the DNA sequence was 40.5%, which is comparable to the 35.8 to 37.1% G+C of the *H. pylori* genome (21).

Predicted polypeptides. *abcA* predicts a polypeptide of 207 amino acids with a molecular size of 22,796 Da and an isoelectric point (pI) of 6.18, while *abcB* predicts a polypeptide of 91 amino acids with a molecular size of 9,953 Da and a pI of 5.13. *abcC* predicts a polypeptide of 328 amino acids with a molecular size of 36,594 Da and a pI of 8.07. The hydropathy plots (data not shown) indicate that these three polypeptides are most likely soluble proteins. *abcD* predicts a polypeptide of 214 amino acids with a molecular size of 22,818 Da and a pI of 8.54. Hydropathy analysis of the putative AbcD polypeptide reveals a highly hydrophobic protein with four potential transmembrane or membrane-associated domains (data not shown). Each of these domains has a Kyte-and-Doolittle hydrophobicity index of ≥1.6 with a window of 20 amino acid residues, consistent with the profile of an integral membrane protein (34). When the Motifs program was used to identify motifs within the Abc polypeptides, only an ATP-binding domain was found within AbcC (see below). No signal peptides were predicted for any of the putative Abc polypeptides.

Amino acid similarity. When the SwissProt database was searched for proteins with sequences similar to that of the predicted 328-amino-acid AbcC polypeptide, several members of the ATP-binding protein family were identified. When aligned with sequences having >25% amino acid identity, the sequence of the AbcC polypeptide had the highest homology to that of an *E. coli* ATP-binding protein (unpublished data; GenBank accession no. P30750): 187 of 201 residues aligned, 114 were identified as exact matches, 28 conservative replacements were found, and one gap of 3 amino acid residues was introduced (56% amino acid identity and 70% amino acid similarity [identical residues plus conservative replacements]). Other proteins identified include the glutamine transport ATP-binding protein (GlnQ) of *Bacillus stearothermophilus* (32% amino acid identity and 52% similarity) and the glycine betaine–L-proline transport ATP-binding protein (ProV) of *E. coli* (30% amino acid identity and 58% similarity over the regions that overlap) (22, 59).

ATP-binding proteins representative of the ABC transporter family of which *nikD* is a member have conserved amino acid sequences characteristic of these proteins (9), including the glycine-rich Walker motif A and Walker motif B separated by a putative helical domain which contains another conserved region (linker peptide) at the junction of the helical domain and Walker motif B (Fig. 3) (49, 56). These motifs are components of a nucleotide-binding fold.

Searches of databases for proteins with homology to the 214-amino-acid AbcD polypeptide yielded one potential homolog and several less-homologous entries (Table 3). A hypothetical 23.3-kDa protein in the *rcsF-rrnH* intergenic region (unpublished data; GenBank accession no. P31547) of *E. coli* had the strongest amino acid identity, with 175 residues aligned, 83 exact matches, and 56 conservative replacements

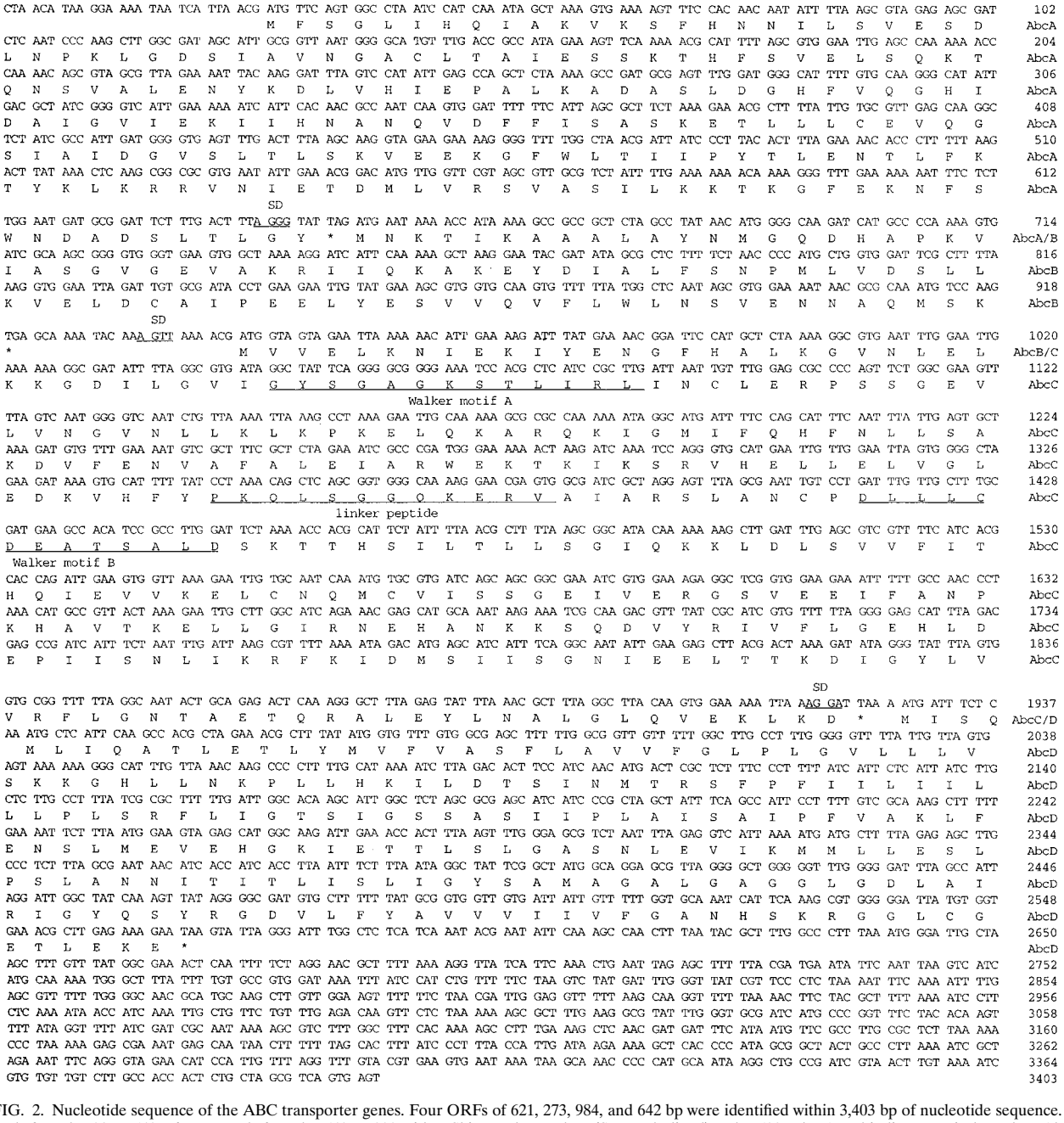

FIG. 2. Nucleotide sequence of the ABC transporter genes. Four ORFs of 621, 273, 984, and 642 bp were identified within 3,403 bp of nucleotide sequence. *abcA* extends from bp 28 to 649; *abcB* extends from bp 649 to 922 with a Shine-Dalgarno box (SD; underlined) at bp 639. The ATP-binding protein homolog, AbcC, is encoded by DNA extending from bp 943 to 1927 and is preceded by an SD sequence at position 933. Walker A and B motifs and the linker region of the AbcC polypeptide are underlined. *abcD* has an SD sequence at position 1919 and extends from bp 1927 to 2569.

with no gaps introduced (43% identity and 72% similarity for aligned sequences). The next most homologous polypeptide was a hypothetical 22.7-kDa protein from *Saccharomyces cerevisiae* (24% amino acid sequence identity) (55). No functions have been assigned to these homologs.

AbcA and AbcB polypeptides were not highly homologous to other database entries. The AbcA polypeptide was found to be most homologous to the riboflavin synthase alpha chain (EC 2.5.1.9) of *Bacillus subtilis* with 31% amino acid sequence identity (47), while the AbcB polypeptide had 21% identity with a small portion of an oligopeptide-binding protein from *Streptococcus pneumoniae* (Table 3) (1).

Construction of *abc* allelic exchange mutants. To assess the contribution of the *abc* gene cluster to urease activity, *abcC* and *abcD* were subjected to allelic exchange mutagenesis in *H. pylori*. To facilitate selection of a cloning site for insertional mutagenesis of *abcC* and *abcD*, a 2.4-kb fragment of pZAP9-1F was amplified and subcloned into the unique *Srf*I restriction site of pCR-Script SK(+) to create clone pABC103 (Fig. 1). A 1.4-kb *Eco*RI fragment containing the *aphA* kanamycin resis-

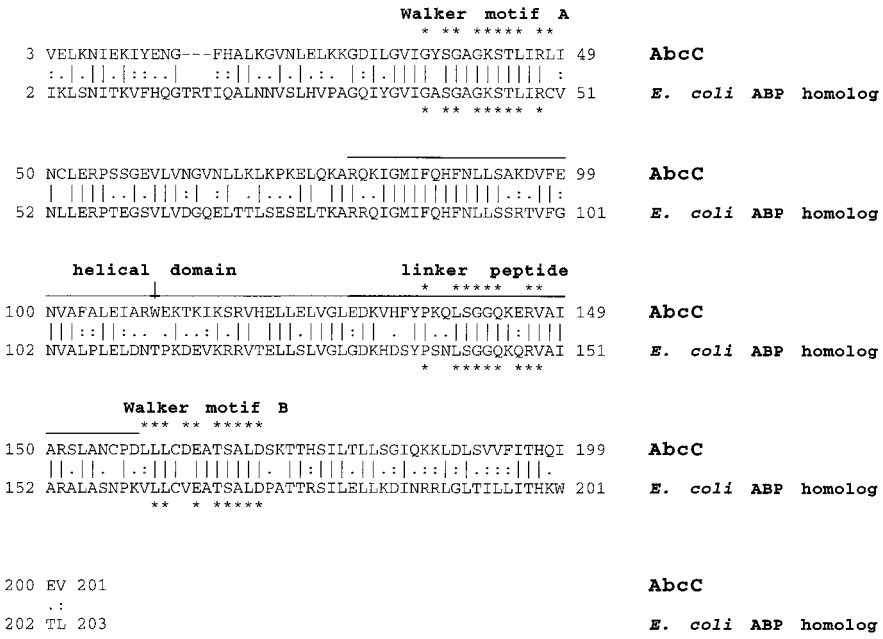

FIG. 3. Sequence alignment and conserved regions within AbcC polypeptide. Alignment of AbcC with its *E. coli* ATP-binding protein (ABP) homolog is shown. Analysis of the predicted 36.6-kDa polypeptide encoded by *abcC* reveals sequence motifs characteristic of ABC transporters. Walker motifs A and B, shown in bold, are linked through a helical domain (line) containing a highly conserved linker peptide (56). These domains are postulated to form a nucleotide-binding fold where ATP is bound and hydrolyzed during transport (9). Identical residues (|), more-conserved residues (:), and less-conserved residues (.) are indicated. Residues conserved among ATP-binding proteins are also indicated (*).

tance cassette from pHP1 was then rendered blunt ended and cloned into the unique *Sty*I restriction site within *abcC* of pABC103 to make pABC203 (Fig. 1). The blunt-ended 1.4-kb *aphA* cassette was also cloned into the *Nhe*I restriction site within *abcD* of pABC103 to make pABC204 (site shown in Fig. 1). Allelic exchange mutants were then constructed by electroporation of plasmids pABC203 and pABC204 into *H. pylori* UMAB41, which was then plated on blood agar containing kanamycin (30 μg/ml). Kan^r colonies were confirmed as *H. pylori* based upon negative Gram stain, spiral morphology, positive oxidase, catalase, and urease activities, and PCR amplification of *H. pylori ureA* and *ureB* urease structural subunit genes on a 2.4-kb fragment by use of specific primers (data not shown). Isolated Kan^r colonies were selected and screened for a double-crossover chromosomal mutation of *abcC* or *abcD* by PCR and Southern blot analysis.

PCR analysis of chromosomal DNA from parent *H. pylori* UMAB41 and the *abcC* mutant, designated HP-ABC3, demonstrated that the *aphA* Kan^r cassette indeed resides within *abcC* (Fig. 4A). Primers, used to amplify a 1.2-kb fragment containing the *abcC* ORF and which also flank the *Sty*I cloning site for the Kan^r cassette, amplified the expected 1.2-kb region of plasmid pABC103 and chromosomal UMAB41 DNA. When plasmid pABC203 and HP-ABC3 chromosomal DNA were used as templates, amplification resulted in a PCR product of 2.6 kb, consistent with the insertion of the 1.4-kb *aphA* cassette. Similar results were obtained with PCRs carried out with UMAB41 chromosomal DNA and the *abcD* mutant, designated HP-ABC4, by use of primers which flank the *Nhe*I cloning site for the Kan^r cassette. These primers amplified the expected 1.3-kb fragment from pABC103 and UMAB41 chromosomal DNA, while the use of pABC204 and HP-ABC4 chromosomal DNA as templates resulted in a PCR product of 2.7 kb, consistent with the insertion of the 1.4-kb *aphA* Kan^r cassette (data not shown).

Southern blot analysis of PCRs using two separate probes, one for *abcC* (Fig. 4B, left panel) and one for the *aphA* Kan^r cassette (Fig. 4B, right panel), confirms that the *aphA* Kan^r cassette is inserted into *abcC*. As expected, the *abcC* probe hybridized to the 1.2-kb PCR products of plasmid pABC103 and chromosomal UMAB41 DNA and also to the 2.6-kb *aphA*-interrupted *abcC* in plasmid pABC203 and HP-ABC3 chromosomal DNA (Fig. 4B, left panel). However, the *aphA* Kan^r cassette probe hybridized only to the 2.6-kb PCR products of plasmid pABC203 and HP-ABC3 chromosomal DNA (Fig. 4B, right panel). Both probes also hybridized to a 1.6-kb band that was determined to be the result of false priming within the Kan^r cassette. (Computer analysis revealed a potential priming

TABLE 3. Predicted genes and homologs of the *H. pylori abc* gene cluster

Gene	No. of nucleotides (bp)	No. of amino acids	Molecular size (kDa)	% Amino acid identity	%5 Amino acid similarity	Homologous protein	Reference or GenBank accession no.
abcA	621	207	22.7	31	52	*B. subtilis* riboflavin synthase	47
abcB	273	91	9.9	21	40	*S. pneumoniae* oligopeptide-binding protein	1
abcC	984	328	36.6	56	70	*E. coli* ATP-binding protein	P30750
abcD	642	214	22.8	43	71	*E. coli* hypothetical intergenic protein	P31547

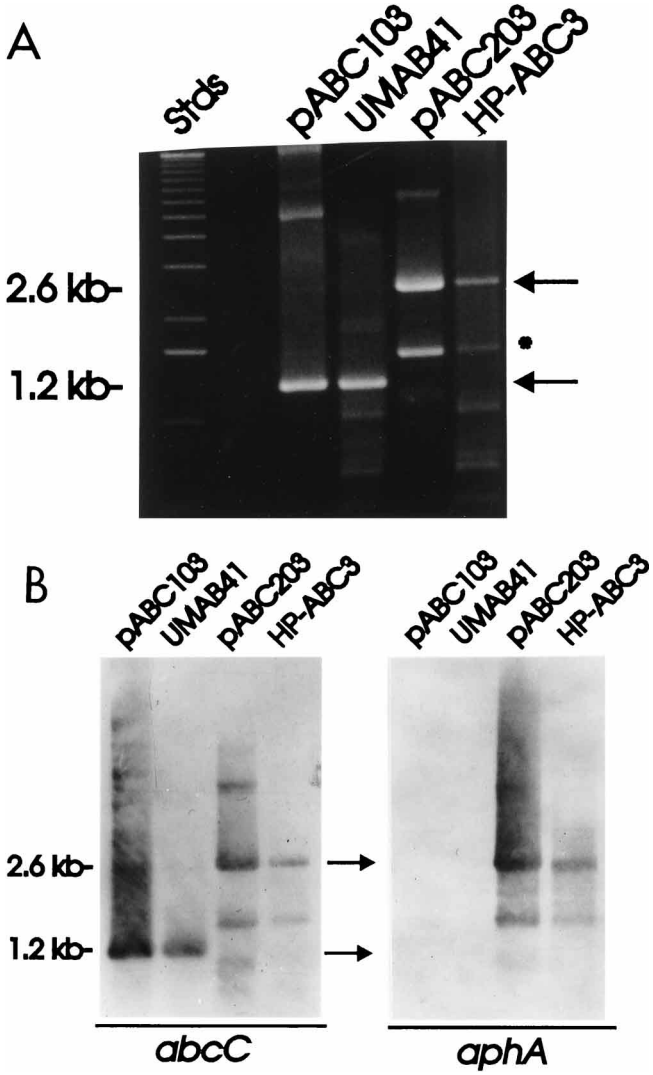

FIG. 4. PCR and Southern blot analysis of *H. pylori* UMAB41 and allelic exchange mutant HP-ABC3. (A) PCR. Oligonucleotide primers flanking *abcC* were used to PCR amplify fragments from plasmid or genomic DNA templates. Stds, 1-kb ladder DNA standards. The size of the fragment predicted to be amplified from the wild-type *abcC* gene, 1.2 kb, is indicated. DNA templates are indicated above the lanes. pABC103 carries the wild-type *abcC* gene, strain UMAB41 is the wild-type clinical isolate, pABC203 carries the *abcC* gene interrupted with the Kan^r cassette, and HP-ABC3 is the AbcC-deficient allelic exchange mutant. The size of the fragment predicted for the *abcC* gene interrupted with the 1.4-kb Kan^r cassette, 2.6 kb, is indicated. The large dot between the two arrows indicates a secondary band possibly generated by false priming within *aphA* sequences (see text). (B) Southern blot of the agarose gel depicted in panel A. (Left) The blot probed with a restriction fragment derived from *abcC*; (right) the blot probed with a restriction fragment derived from *aphA* (the kanamycin resistance cassette). Arrows between the two panels correspond to the 1.2-kb and 2.6-kb markers.

site within the Kan^r cassette having 12 of 18 identical matches that could generate a fragment of this size.) Similar results were obtained with a probe of *abcD* and the *aphA* cassette to probe Southern blots of the PCRs specific for *abcD* (no additional band caused by false priming). We concluded that the Kan^r cassette was inserted within *abcC* in the HP-ABC3 chromosome and within *abcD* in the HP-ABC4 chromosome.

Construction of *nixA-abcC* double mutant. Since we reasoned that NixA and the Abc system provide alternate pathways for nickel uptake, it would follow that a double mutation in both *nixA* and an *abc* gene would abolish nickel uptake and greatly reduce urease activity. To accomplish this, a 1.5-kb *Sma*I fragment containing a *cat* chloramphenicol resistance cassette from pSKCAT4 was rendered blunt ended and cloned into the unique *Pfl*MI restriction site within *nixA* of pUEF202 to make pUEF411. This construct was then electroporated into UMAB41 and HP-ABC3, which was then plated on blood agar containing either chloramphenicol (20 μg/ml) or chloramphenicol and kanamycin (30 μg/ml). Cm^r colonies of the electroporated UMAB41 or Cm^r Kan^r colonies arising from electroporated HP-ABC3 were confirmed as *H. pylori* as described above. Cm^r (UMAB41) and Cm^r Kan^r (HP-ABC3) colonies were selected and screened for a double-crossover mutation of *nixA* by PCR and Southern blot analysis.

Primers flanking the *Pfl*MI cloning site amplified the expected 517-bp fragment from plasmid pUEF202 and parent UMAB41 and HP-ABC3 chromosomal DNA. When plasmid pUEF411, chromosomal DNA from the *nixA* mutant, designated HP-NIXA, and the double mutant, designated HP-ABC3N, were used as templates, amplification resulted in a PCR product of 2.0 kb, consistent with the insertion of the *cat* cassette (data not shown).

Southern blot analysis of PCRs using a probe for the *cat* cassette confirmed the insertion of the *cat* gene within *nixA*. This probe did not bind to PCR products arising from pUEF202 plasmid and UMAB41 or HP-ABC3 chromosomal DNA templates as expected; however, the *cat* probe did hybridize to the 2.0-kb PCR products of plasmid pUEF411 and chromosomal HP-NIXA and HP-ABC3N (the *abcC-nixA* mutant) DNA templates (data not shown). We concluded that the *cat* cassette was successfully inserted into the HP-NIXA and HP-ABC3 chromosomes.

Urease activity. To determine whether the mutations in the *abc* gene cluster affected urease activity, urease assays of soluble protein were done with the phenol red spectrophotometric assay. The results of multiple independent assays (Fig. 5) show a statistically significant difference ($P < 0.0001$) between the mean urease activity (±standard deviation) of wild-type *H. pylori* UMAB41 (228 ± 92 μmol of NH_3/min/mg of protein) and that of *abcD* mutant HP-ABC4 (27 ± 31 μmol of NH_3/

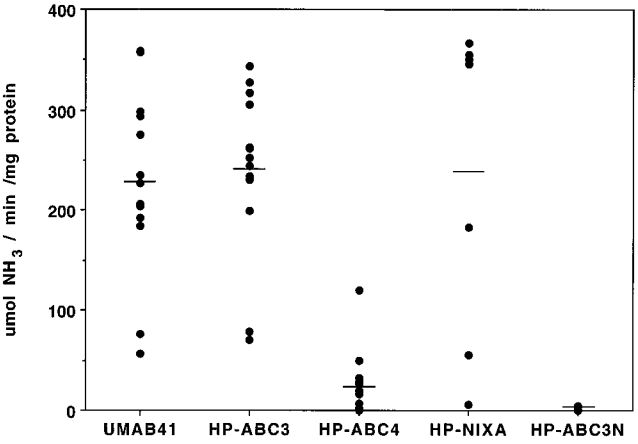

FIG. 5. Urease activity of UMAB41 and *abc* mutants. Parent and mutant strains were washed in phosphate-buffered saline and lysed by passage through a French pressure cell. Insoluble protein and membrane were removed by centrifugation. Soluble protein (10 μg) from each strain was assayed for urease activity by the phenol red spectrophotometric method. Urease activity (micromoles of NH_3 liberated per minute per milligram of protein) was calculated by use of the linear portions of the curve. Each datum point represents an independent assay ($n = 7$ for HP-NIXA; $n = 13$ for all other strains).

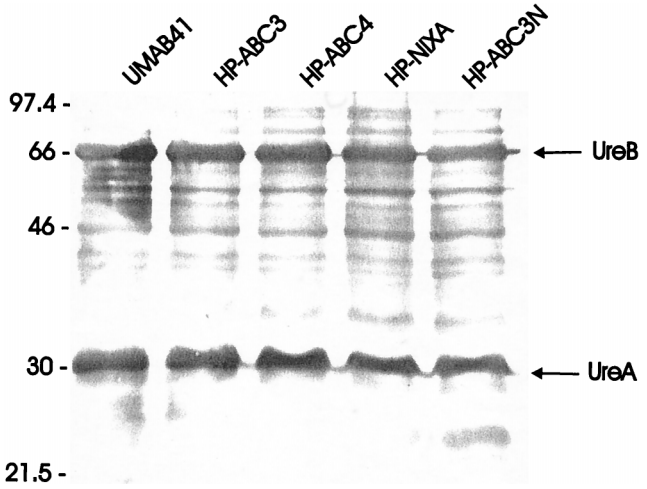

FIG. 6. Western blot analysis of soluble protein from *H. pylori* UMAB41 and *abc* mutants. Soluble protein (5 μg) from UMAB41, HP-ABC3, HP-ABC4, HP-NIXA, and HP-ABC3N was run through an SDS–12% polyacrylamide gel and transferred onto an Immobilon P membrane. UreA and UreB proteins were detected by incubation of the blot with rabbit anti-UreA and anti-UreB polyclonal antisera followed by goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase as a secondary antibody. Protein standards with known molecular sizes (given in kilodaltons to the left of the gel) were phosphorylase *b* (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (46 kDa), carbonic anhydrase (30 kDa), and trypsin inhibitor (21.5 kDa).

min/mg of protein). The mutation resulted in an 88% decrease in urease activity in the mutant *H. pylori* strain. Urease activities of *H. pylori* strains containing single mutations in *abcC* (HP-ABC3) or *nixA* (HP-NIXA) were 240 ± 85 and 237 ± 135 μmol of NH_3/min/mg of protein, respectively, and did not differ significantly from that of the wild-type parent UMAB41. The double mutation of both *abcC* and *nixA* in strain HP-ABC3N, however, resulted in a reduction of urease activity to 1.08 ± 1.43 μmol of NH_3/min/mg of protein ($P < 0.0001$), a decrease of greater than 99% in urease activity.

Apourease synthesis. We hypothesized that the mutations in the *abc* gene cluster would not directly affect production of urease apoenzyme. To test this hypothesis, soluble protein from cell lysates of the wild-type (UMAB41) and mutant strains was subjected to SDS-PAGE and Western blot analysis (Fig. 6). UreA and UreB urease subunits were visible in the Coomassie blue-stained gel at the expected sizes of 29.5 and 66 kDa, respectively (data not shown). These polypeptides were also recognized on a Western blot by use of rabbit antisera raised individually against both UreA and UreB subunits and appear to be produced in equal amounts in the wild-type and mutant strains of *H. pylori*, indicating that production of urease subunits UreA and UreB is unaffected by mutation of the *abc* gene cluster.

Nickel incorporation into urease. To ascertain whether the mutation in *abcC* affected the incorporation of Ni^{2+} into the urease active site, immunoprecipitation experiments using $^{63}NiCl_2$ were performed. Results of three independent experiments (Fig. 7) indicated an apparent reduction in the amount of $^{63}Ni^{2+}$ incorporated into the urease of HP-ABC3 compared to incorporation into urease of UMAB41; however, these differences were not statistically significant.

Nickel transport. The reduction in urease activity in the *abcD* and *abcC-nixA* mutant was likely due to a reduction in nickel ion available for insertion into the enzyme active site rather than a reduction in apoenzyme synthesis. To assess whether this difference was due to reduced uptake of nickel into the cell, nickel transport assays were conducted in triplicate with *H. pylori* UMAB41 and HP-ABC3 by use of $^{63}NiCl_2$ at concentrations ranging from 50 nM to 1 μM. The rate of uptake at each concentration was calculated for wild-type and mutant strains, and the mean rate for the first three (0.5-, 1-, and 2-min) and last three (2-, 5-, and 10-min) time points was determined. Uptake assays were also conducted at 50 and 500 nM for HP-ABC4, HP-NIXA, and HP-ABC3N. The results of unpaired, two-tailed *t* tests comparing the mean rates of nickel uptake for strains UMAB41 and all *abc* and *nixA* mutants at identical concentrations and time spans indicated that there were no statistically significant differences between $^{63}Ni^{2+}$ uptake of UMAB41 and that of any mutant at any of the concentrations of $^{63}NiCl_2$ over any time spans measured under these specific uptake conditions. These data, however, were not highly reproducible and varied considerably between experiments. This variability is in stark contrast to highly reproducible uptake curves obtained with *E. coli* SE5000(pUEF202) expressing *nixA* (41).

Catalase and oxidase assays. To determine whether the effect of the *abcC* mutation affected the activities of other enzymes, catalase and oxidase activities were also measured. Both of these assays are commonly used to identify *H. pylori* in culture. No reduction in catalase activity was noted in mutant HP-ABC3 (1,759 ± 352 μmol of H_2O_2/min/mg of protein) compared to that of UMAB41 (916 ± 229 μmol of H_2O_2/min/mg of protein). These data represent mean values of three independent measurements ± standard deviations.

Oxidase activity, measured by noting the appearance and intensity of the blue oxidized form of tetramethyl-*p*-phenylenediamine on filter paper when bacterial cells were spotted onto this indicator, was unaffected by the mutation in *abcC* (Fig. 8).

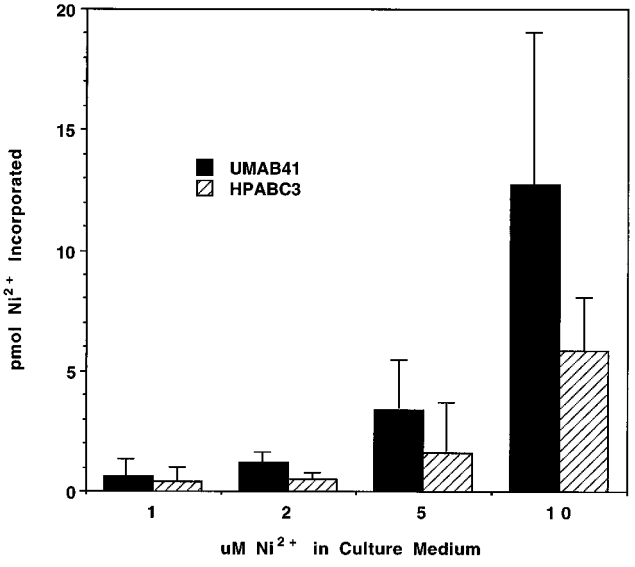

FIG. 7. Nickel ion incorporated into urease synthesized by *H. pylori* UMAB41 and HP-ABC3. *H. pylori* strains were grown under microaerobic conditions in Mueller-Hinton broth supplemented with 4% horse serum and containing $^{63}NiCl_2$ at concentrations ranging from 1 to 10 μM. Soluble protein (100 μg) from French press-lysed bacteria was subjected to immunoprecipitation with rabbit polyclonal antisera directed against *H. pylori* UreB, the large structural subunit of urease. The amount of $^{63}Ni^{2+}$ incorporated into the precipitated protein was determined by liquid scintillation.

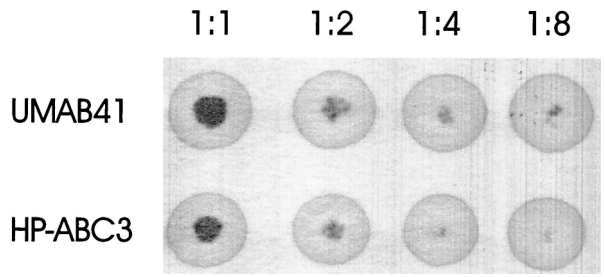

FIG. 8. Oxidase activity of *H. pylori* UMAB41 and HP-ABC3 (*abcC* mutant). Bacterial suspensions (5 μl) at the dilutions indicated were spotted onto filter paper along with a drop of oxidase reagent. A dark color is indicative of a positive oxidase reaction. *H. pylori* UMAB41, wild-type strain; HP-ABC3, *abcC* mutant.

DISCUSSION

We have described a new gene cluster, designated *abcABCD*, that is necessary for full catalytic activity of the urease of *H. pylori*. This gene cluster appears to encode an ABC transport system, the first described for *H. pylori*. The presence of the intact transporter is necessary for complete incorporation of nickel ions into the active site of the urease metalloenzyme. Catalytic activity of urease, in turn, requires this metal ion incorporation. Four ORFs, transcribed in the same direction, predict polypeptides of 22.7 (AbcA), 9.9 (AbcB), 36.6 (AbcC), and 22.8 (AbcD) kDa and bear resemblance to typical ABC transporter systems. This includes an ATP-binding polypeptide and an integral cytoplasmic membrane protein (16). The 36.6-kDa polypeptide, AbcC, contains highly conserved amino acid sequences, including Walker A and B motifs joined by a helical region containing a characteristic linker peptide. In addition, AbcD is a highly hydrophobic protein with four potential transmembrane domains which may form the channel for the transported substrate. Putative polypeptides AbcA and AbcB were not highly homologous to any proteins in the available databases; however, the lack of sequence similarity of AbcA and AbcB with other ABC transporter components is not unexpected since comparisons of amino acid sequences among different ABC transmembrane proteins reveal little or no similarity (26). Our data indicate that there are no significant ORFs within 1 kb downstream of *abcD*; however, it remains to be seen whether related genes exist immediately upstream of *abcA*.

The ABC (ATP-binding cassette) family of transporters is common among prokaryotes; several examples also exist in eukaryotes. They can act as importers or exporters with substrates as diverse as alpha-hemolysin (*E. coli*, *Proteus vulgaris*, and *Morganella morganii*), subtilin (*B. subtilis*), capsular polysaccharides (*E. coli*, *Haemophilus influenzae*, and *Neisseria meningitidis*), heme (*Bradyrhizobium japonicum*), hydrophobic drugs (*Streptomyces peucetius* and eukaryotes), and cations (*Rhizobium leguminosarum*, *N. meningitidis*, and *Serratia marcescens*), to name a few (16). Some of these transporters are accessory virulence factors as they act to export toxins and antibiotics into the extracellular medium (8, 20, 45), while others import amino acids, ions, and various other nutrients required for normal cell metabolism (12, 27, 43).

To identify a phenotype and to investigate the role of the Abc transport system, we constructed allelic exchange mutants by use of a Kan^r cassette inserted at different sites within the *abc* gene cluster. Mutant HP-ABC4 (*abcD* mutant) and double mutant HP-ABC3N (*abcC-nixA* mutant) demonstrated significant reduction in urease activities despite the fact that similar amounts of urease apoenzyme were synthesized by wild-type and mutant strains. Direct transport of nickel into the cell would be one possible mechanism; however, we were unable to measure differences in nickel transport between *H. pylori* UMAB41 and mutant HP-ABC3 under the conditions tested. There are other possible mechanisms by which the transporter could be affecting catalytic activity, such as importation of a cofactor required by urease accessory proteins for insertion of nickel into the enzyme active site.

Initial interest in *abcABCD* was raised because of the limited homology of AbcC to NikD of *E. coli*, which is a component of a nickel-transporting ABC transport system (43). We hypothesized that the *abcABCD*-encoded system might play a similar role in providing nickel to urease, an important virulence factor of *H. pylori*. Although our data only circumstantially implicate the function of these proteins as importer, we can hypothesize the requirements of an ABC transport system for *H. pylori*. First, the gram-negative nature of this organism makes it likely that an accessory factor is necessary to ferry the substrate through the periplasm and present it to the membrane-resident transporter. This is almost certainly the case if the transporter is involved in solute uptake, since all ABC importers identified to date have a periplasmic substrate-binding protein, whereas no exporter-associated periplasmic substrate-binding proteins have been identified (26). If the system acts as an exporter, it may require another protein to facilitate crossing of the outer membrane as in the hemolysin export secretion system of *E. coli* (17).

The presence of Walker motifs alone does not identify AbcC as an ABC protein since many nucleotide-binding proteins contain these regions. Sequence identity with the *E. coli* ABC homolog extends beyond these motifs to the entire domain of the protein, distinguishing AbcC as a member of the ABC family (26). Of the four putative polypeptides identified to date, only AbcD could potentially form the membrane-spanning substrate channel based on hydropathy analysis. In the absence of another Abc-associated integral membrane protein, the hydrophobic AbcD may form a homodimer, a common conformation of ABC transmembrane proteins, consisting of eight transmembrane domains. This would represent the fewest transmembrane segments yet reported for an ABC system. *abcA* or *abcB* may encode accessory proteins that facilitate the transport process.

Clearly, the presence of the *abc* system is necessary for full catalytic activity of urease; however, this activity must be viewed in the context of other *H. pylori* proteins that have been implicated in production of a catalytically active urease. NixA, a high-affinity nickel transporter, has been identified as an integral membrane protein capable of importing nickel ions in an energy-dependent fashion across the cytoplasmic membrane (41). Mutation of the gene encoding this protein can reduce nickel transport and thus urease activity in some strains, probably due to a reduction in the number of nickel ions inserted into the apoenzyme during assembly (5). Also, a P-type ATPase for which mutations dramatically reduce urease activity in *H. pylori* has been identified (38). In addition, a histidine-rich metal-binding protein, Hpn, has been found to bind a number of different metal ions, including nickel, within the cell (19). Its role, however, in production of active enzyme is not clear. Finally, HspA, which bears a histidine tail also capable of binding nickel ions, has also been implicated in production of a catalytically active urease (31, 32). In some instances, it appears that sufficient nickel ion can be accumulated even when one system has been mutated. For example, the *abcC* mutant retained active urease as did the *nixA* mutant under the conditions tested. It is likely that one transport system complemented the loss of function of the other system.

Mutation of both *abcC* and *nixA*, however, understandably abolished nickel transport and thus urease activity.

At this writing, no ABC transporters have been described for *H. pylori*, and while the data to date do not indicate that this ABC transport system is directly analogous to the Nik system of *E. coli*, they certainly suggest that this gene cluster is a member of the same family of ABC transporters. We conclude that we have identified a novel ABC transport system in *H. pylori*, the activity of which contributes to synthesis of a catalytically active urease enzyme. Although it cannot be demonstrated directly that this system transports nickel ions into *H. pylori*, neither can this possibility be excluded. Mutation of this transport system, nevertheless, reduces urease activity by decreasing incorporation of Ni^{2+} into the enzyme active site. Our future research will be directed toward identification of the substrate and function of the ABC system, its dependence on ATP for transport, and the specific mechanism by which it serves to enhance the urease activity of *H. pylori.*

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