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NikR Mediates Nickel-Responsive Transcriptional Induction of Urease Expression in *Helicobacter pylori*

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The important human pathogen *Helicobacter pylori* requires the abundant expression and activity of its urease enzyme for colonization of the gastric mucosa. The transcription, expression, and activity of *H. pylori* urease were previously demonstrated to be induced by nickel supplementation of growth media. Here it is demonstrated that the HP1338 protein, an ortholog of the *Escherichia coli* nickel regulatory protein NikR, mediates nickel-responsive induction of urease expression in *H. pylori*. Mutation of the HP1338 gene (*nikR*) of *H. pylori* strain 26695 resulted in significant growth inhibition of the *nikR* mutant in the presence of supplementation with $NiCl_2$ at ≥100 μM, whereas the wild-type strain tolerated more than 10-fold-higher levels of $NiCl_2$. Mutation of *nikR* did not affect urease subunit expression or urease enzyme activity in unsupplemented growth media. However, the nickel-induced increase in urease subunit expression and urease enzyme activity observed in wild-type *H. pylori* was absent in the *H. pylori nikR* mutant. A similar lack of nickel responsiveness was observed upon removal of a 19-bp palindromic sequence in the *ureA* promoter, as demonstrated by using a genomic *ureA*::*lacZ* reporter gene fusion. In conclusion, the *H. pylori* NikR protein and a 19-bp operator sequence in the *ureA* promoter are both essential for nickel-responsive induction of urease expression in *H. pylori*.

The gram-negative human pathogen *Helicobacter pylori* colonizes the mucus overlaying the gastric epithelium, leading to chronic gastritis that can subsequently develop into peptic ulcer disease and gastric cancer (12). Approximately half of the world's population is colonized by *H. pylori*, constituting a major public health problem (12).

One of the factors required for gastric colonization by *H. pylori* is its urease enzyme (13, 14, 37). Urea hydrolysis by urease yields ammonia and bicarbonate, and these products have important functions in *H. pylori* colonization and infection. Ammonia contributes to acid resistance by neutralizing the microenvironment of the bacterium (8, 27), serves as a nitrogen source (10), is essential for chemotactic behavior (25), and causes damage to the gastric epithelium (30). Bicarbonate also contributes to the virulence of *H. pylori*, as it protects against the bactericidal activity of peroxynitrite, a nitric oxide metabolite (20). Urease is also involved in the resistance of *H. pylori* to opsonization and phagocytosis and in the adherence of *H. pylori* to epithelials cells, although these functions do not depend on enzymatic activity or ammonia production via urease (21, 22, 26).

H. pylori produces large amounts of urease, and it has been estimated that up to 10% of the total protein content of *H. pylori* consists of urease (3). Active urease is a multimeric enzyme that consists of six UreA and six UreB subunits and 12 Ni^{2+} ions functioning as a cofactor (19, 23). The 27-kDa UreA and 62-kDa UreB urease subunits are encoded by the *ureA* and *ureB* genes, respectively, which are followed by a second operon encoding the UreIEFGH accessory proteins (23). The UreEFGH accessory proteins are involved in assembly and activation of urease, while the UreI protein probably functions as an acid-activated urea transporter (23, 27, 36).

Transcription of the *H. pylori* urease gene cluster occurs from two promoters, one upstream of the *ureA* gene and one in the intergenic region between *ureB* and *ureI* (1, 29). Transcription from these two promoters and subsequent pH-dependent differential mRNA decay lead to the formation of *ureAB*, *ureABIE′*, *ureIE′*, and *ureF′GH* mRNAs (1). Urease production in other ureolytic bacteria is known to be regulated by changes in environmental conditions, such as pH, urea availability, nitrogen availability, or growth phase (5). Uniquely, not only urease activity but also the expression of urease in *H. pylori* is regulated by the availability of the nickel cofactor (33). Nickel supplementation of brucella medium resulted in a 4-fold induction of urease expression at the protein level and a 12-fold induction of urease enzyme activity. The regulatory system mediating this nickel-responsive induction of urease expression has not yet been described (33).

Nickel-responsive regulation of gene expression has been observed in several bacteria, but the molecular mechanisms have been studied only for a few systems (15). One of the best-studied examples is the regulation of expression of nickel uptake in *Escherichia coli*, where the expression of the nickel transport operon *nikABCDE* is controlled by the NikR protein (6, 7, 9). NikR represses the transcription of the nickel uptake genes by binding to an operator sequence located in the target promoter region upon increased cytoplasmic nickel availability

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TABLE 1. *H. pylori* strains and plasmids used in this study

Strain or plasmid	Genotype or relevant characteristics	Reference or source
H. pylori strains		
26695	Wild-type strain	32
26695*nikR*	26695 *nikR*::Km^r	This study
1061	Wild-type strain	17
AV433	1061 *ureA*::*lacZ* Km^r	33
1061BJD3.8	1061 *ureA*(Δ−50/−112)::*lacZ* Km$^{r a}$	This study
1061BJD3.9	1061 *ureA*(Δ−50/−90)::*lacZ* Km^r	This study
1061BJD3.10	1061 *ureA*(Δ−50/−70)::*lacZ* Km^r	This study
Plasmids		
pAV348	pBluescript derivative containing a 1.5-kb DNA fragment containing the *H. pylori* 1061 *nikR* gene	This study
pAV364	pAV348 with the Km^r cassette of pJMK30 (34) inserted in the unique *Sph*I restriction site	This study
pBW	*H. pylori* promoter-probe vector; Km^r	11
pBJD3.3	pBW derivative containing the *H. pylori* 1061 *ureA* promoter fused to the *lacZ* gene of pBW; Km^r	33
pBJD3.8	pBJD3.3 derivative [*ureA*(Δ−50/−112)::*lacZ*]; Km^r	This study
pBJD3.9	pBJD3.3 derivative [*ureA*(Δ−50/−90)::*lacZ*]; Km^r	This study
pBJD3.10	pBJD3.3 derivative [*ureA*(Δ−50/−70)::*lacZ*]; Km^r	This study

[a] *ureA*(Δ−50/−112), *ureA* lacking the sequences from positions −50 to −112.

(6, 7, 9, 15), in a fashion similar to that of the family of Fur metalloregulatory proteins (16).

Urease activity is essential for gastric colonization by *H. pylori* (13, 14, 37), and its regulation is probably also necessary for successful colonization. *H. pylori* contains a gene (2, 32) which encodes a protein (HP1338 or JHP1257) homologous to the *E. coli* NikR protein (6, 7, 9). Here we report on the role of the *H. pylori* NikR ortholog in the nickel-responsive induction of urease expression and activity and identify a nickel-responsive operator sequence in the urease promoter.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions. The *H. pylori* strains and plasmids used in this study are listed in Table 1. *H. pylori* was routinely maintained on Columbia agar plates supplemented with 7% saponin-lysed horse blood, 0.004% triphenyltetrazolium chloride (Sigma), and Dent selective supplement (Oxoid) (4) at 37°C under microaerobic conditions (10% CO_2, 5% O_2, and 85% N_2). Broth cultures were grown under the same conditions in brucella broth (Difco) supplemented with 3% newborn calf serum (Gibco) (BBN). Nickel chloride (Sigma) was filter sterilized and used at various concentrations. *E. coli* strains DH5α MCR (Gibco) and ER1793 (New England Biolabs) were grown aerobically at 37°C in Luria-Bertani medium (28). When appropriate, growth media were supplemented with ampicillin (100 μg/ml), kanamycin (20 μg/ml), or chloramphenicol (10 μg/ml).

Recombinant DNA techniques. Restriction enzymes and DNA-modifying enzymes were used according to the manufacturer's instructions (New England Biolabs). Standard protocols were used for the manipulation of DNA and the transformation of *E. coli* (28) and *H. pylori* (4). Plasmid DNA was prepared by using Qiaprep spin columns (Qiagen), and PCR was carried out by using *Taq* polymerase (Promega).

Construction of an *H. pylori nikR* mutant. The region containing the *H. pylori nikR* ortholog and its upstream and downstream sequences was amplified from *H. pylori* strain 1061 by using primers F1337 (5′-TAGAAGAAATTGGCGCGT CA) and 1339R (5′-TCACGCCCATGTCATAGAA). The resulting *nikR* PCR fragment was cloned in pBluescript II SK(−) (Stratagene), resulting in pAV348 (Table 1). The *nikR* coding region in pAV348 was interrupted by insertion of the kanamycin resistance gene from pJMK30 (34) in the unique *Sph*I site, resulting in plasmid pAV364 (Table 1). This plasmid was subsequently used for natural transformation of *H. pylori* 26695, and the kanamycin-resistant colonies isolated were designated 26695 *nikR*. Two colonies derived from independent transformations were tested and gave identical results in all experiments. Correct allelic replacement of the wild-type *nikR* gene with the interrupted version was confirmed by PCR-based analysis (data not shown).

Protein analysis. *H. pylori* cultures were grown in unsupplemented or $NiCl_2$-supplemented BBN for 20 to 24 h at 37°C with moderate shaking to an optical density at 600 nm (OD_{600}) of 0.4 to 0.8, centrifuged at 4,000 × *g* for 10 min at 4°C, and resuspended in ice-cold phosphate-buffered saline to a final OD_{600} of 10. *H. pylori* cells were lysed by sonication for 15 s on ice with an MSE Soniprep 150 set at amplitude 10. Protein concentrations were determined by the bicinchoninic acid method (Pierce) with bovine serum albumin as the standard. Samples containing 15 μg of protein were separated by sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue (28).

Urease activity. The urease activity of fresh lysates was determined by measuring ammonia production from urea hydrolysis with the Berthelot reaction as described previously (33, 35). The amount of ammonia present in the samples was inferred from a standard NH_4Cl concentration curve. Urease activity was expressed as micromoles of urea hydrolyzed per minute per milligram of protein.

***ureA*::*lacZ* transcriptional fusions.** Plasmids pBJD3.3, pBJD3.8, pBJD3.9, and pBJD3.10 were used to test the effect of the palindromic sequence at positions −49 to −67 on the nickel-responsive induction of the *ureA* promoter (Table 1). Plasmids pBJD3.8, pBJD3.9, and pBJD3.10 were all derived from pBJD3.3 (33), which contains the *H. pylori* 1061 *ureA* promoter cloned in front of a promoterless *lacZ* gene (11). The *ureA* promoter was modified by inverse PCR mutagenesis (38), resulting in deletions of sequences upstream of the *H. pylori* 1061 *ureA* transcriptional start site ranging from positions −50 to −70, from positions −50 to −90, and from positions −50 to −112. The *ureA* promoter regions of pBJD3.3, pBJD3.8, pBJD3.9, and pBJD3.10 were sequenced to verify correct removal of the desired sequences and the absence of other nucleotide substitutions or deletions. Transformation of *H. pylori* strain 1061 to kanamycin resistance by pBJD3.3 and its mutant derivatives resulted in integration of the plasmid by single homologous recombination, with the cloned promoter region preceding the *lacZ* reporter gene, whereas the wild-type promoter still preceded the intact urease operon (33). The insertion of the pBJD3.3 vector did not have a major effect on the expression, activity, or nickel induction of urease, as demonstrated previously (33). Transformation of *H. pylori* 1061 with pBJD3.3 and its mutant derivatives resulted in the kanamycin-resistant *H. pylori* strains AV433, 1061BJD3.8, 1061BJD3.9, and 1061BJD3.10 (Table 1). The β-galactosidase activities (in Miller units) (28) of these strains grown in either unsupplemented or nickel-supplemented BBN were determined with lysates from freshly sonicated cells as described previously (33).

Nucleotide sequence accession number. The DNA sequence of the *ureA* promoter of *H. pylori* strain 1061 has been deposited in the GenBank sequence database under accession number AY078177.

RESULTS

Identification of the *H. pylori* NikR ortholog. Analysis of the proteins encoded by the *H. pylori* strain 26695 genome (32) for orthologs of nickel regulatory proteins indicated that the HP1338 protein is homologous to the *E. coli* NikR protein,

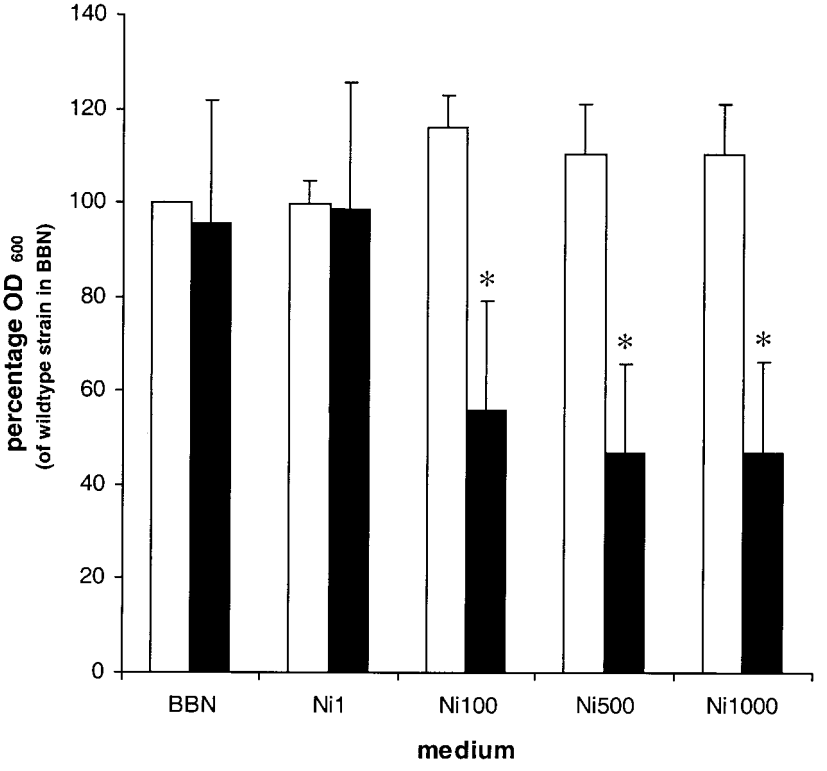


FIG. 1. Insertional inactivation of the *nikR* gene renders *H. pylori* nickel sensitive. Wild-type *H. pylori* strain 26695 and its isogenic *nikR* mutant were grown in unsupplemented medium (BBN) or in BBN supplemented with $NiCl_2$ to final concentrations of 1 μM (Ni1), 100 μM (Ni100), 500 μM (Ni500), and 1,000 μM (Ni1000). Growth was monitored by measuring the OD_{600} 24 h after inoculation. Growth is expressed as a percentage relative to the OD_{600} of the wild-type strain in unsupplemented medium (set at 100%; no error bar). White bars represent wild-type *H. pylori* 26695; blacks bars represent the *nikR* mutant. Results shown are the averages of four independent growth experiments; errors bars denote standard deviations. Asterisks indicate a significant difference in growth between the *nikR* mutant and wild-type *H. pylori* (the *P* value was <0.01, as determined by Student's *t* test).

displaying 30% identity and 68% similarity. Orthologs of other known nickel regulatory proteins, such as the *Ralstonia* Cnr proteins (18, 31), were not apparent. The genetic organization of the genomic region containing the *H. pylori* HP1338 gene (subsequently referred to as *nikR*) is conserved between *H. pylori* strains 26695 and J99 (2, 32). Located downstream of the *nikR* gene is the HP1337 gene, which is annotated as a conserved hypothetical protein (2, 32); the upstream divergent operon encodes an ExbB-ExbD-TonB complex, which in other bacteria is involved in the transport of iron compounds across the outer membrane (24).

Inactivation of the *nikR* gene renders *H. pylori* nickel sensitive. The *nikR* gene of *H. pylori* strain 26695 was interrupted by the insertion of a kanamycin resistance gene, and the effect of the *nikR* mutation on the nickel sensitivity of *H. pylori* was tested by comparing the growth of the wild-type strain and the *nikR* mutant in media with increasing $NiCl_2$ concentrations. The growth of the *nikR* mutant in unsupplemented medium or in medium with 1 μM $NiCl_2$ was not significantly different from that of the wild-type strain (Fig. 1). However, the growth of the *nikR* mutant was clearly affected by medium supplementation with 100 μM $NiCl_2$ or higher (Fig. 1). This result indicates that NikR is required for the nickel resistance of *H. pylori*.

Mutation of *H. pylori nikR* abolishes nickel-responsive induction of urease expression. It was previously reported that medium supplementation with $NiCl_2$ to 100 μM results in an approximately fourfold increase in the expression of the urease subunits UreA and UreB in *H. pylori* strain 26695 (33) (Fig. 2A). The expression of the urease subunits and urease enzyme activity were compared between the wild-type strain and the *nikR* mutant of *H. pylori* 26695 to determine the effect of the *nikR* mutation on the nickel-responsive induction of urease. The expression of the urease subunits UreA and UreB and urease enzyme activity did not differ significantly between the wild-type strain and the *nikR* mutant in unsupplemented BBN (Fig. 2). When BBN was supplemented with 1 μM $NiCl_2$, the levels of urease subunit expression were unchanged (Fig. 2A), but urease enzyme activity was induced approximately threefold in both the wild-type strain and the *nikR* mutant (Fig. 2B). Differences between the wild-type and *nikR* mutant strains were, however, clearly apparent when the medium was supplemented with 100 μM $NiCl_2$ or higher: the *nikR* mutant strain did not show any further induction of urease subunit expression (Fig. 2A) or urease enzyme activity (Fig. 2B), while the wild-type strain clearly showed a significant increase in the expression of the urease subunits UreA and UreB (Fig. 2A) as well as in urease enzyme activity (Fig. 2B).

Identification of a nickel-responsive operator sequence in

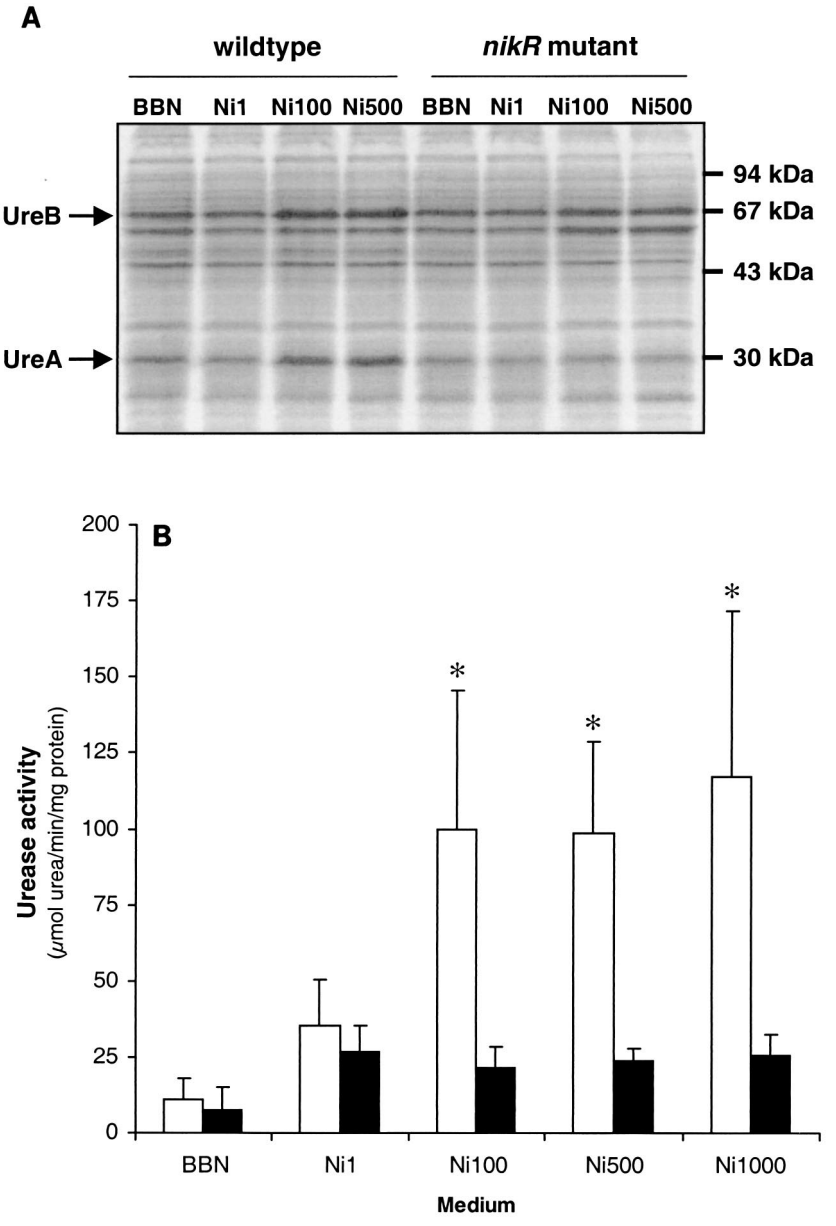
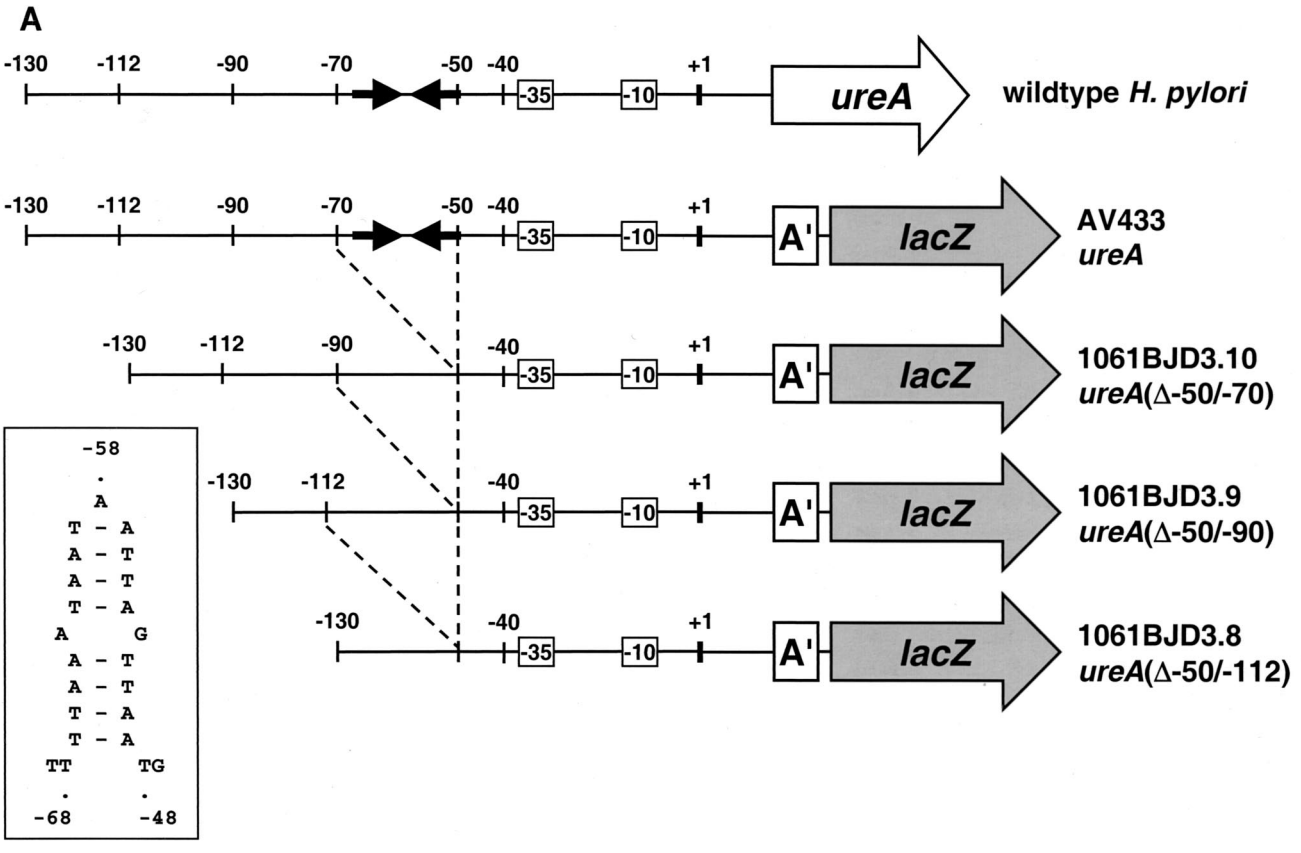


FIG. 2. Insertional inactivation of the *H. pylori nikR* gene inhibits nickel-responsive induction of urease expression and activity. Wild-type *H. pylori* 26695 and its isogenic *nikR* mutant were grown in unsupplemented medium (BBN) or in BBN supplemented with $NiCl_2$ to final concentrations of 1 μM (Ni1), 100 μM (Ni100), 500 μM (Ni500), and 1,000 μM (Ni1000). (A) Changes in urease expression as monitored by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Coomassie brilliant blue staining. *H. pylori* strains, UreA and UreB proteins, and relevant molecular mass markers are indicated. (B) Urease activity of the wild-type strain and the *nikR* mutant in unsupplemented and nickel-supplemented media. White bars represent wild-type *H. pylori* 26695; black bars represent the *nikR* mutant. Results shown are the averages of four independent growth experiments; error bars denote standard deviations. Asterisks indicate a significant difference in urease activity between wild-type *H. pylori* and the *nikR* mutant (the *P* value was <0.02, as determined by Student's *t* test).

the *ureA* promoter. Nickel-responsive induction of urease expression in *H. pylori* is mediated at the transcriptional level via the *ureA* promoter (33). Metal-responsive regulatory proteins, such as NikR, often mediate their regulatory function by binding to palindromic operator sequences located in the promoter regions of the target genes (7, 16). The presence of a palindromic sequence in the *ureA* promoter was described previously, at positions −49 to −67 relative to the transcriptional start site of the *ureA* gene (Fig. 3A) (29). An *H. pylori* chromosomal *ureA*::*lacZ* reporter gene fusion in *H. pylori* strain 1061 (17) was used to assess the role of this palindrome in the nickel-responsive induction of urease expression (33). The wild-type promoter was compared with three mutated versions, which lacked the sequences from positions −50 to −70 (Δ−50/−70), from positions −50 to −90 (Δ−50/−90), or from positions −50 to −112 (Δ−50/−112), respectively. Effectively, all mutants lacked the palindromic sequence at positions −49 to −67 with no effect on the −35 and −10 regions (Fig. 3A).



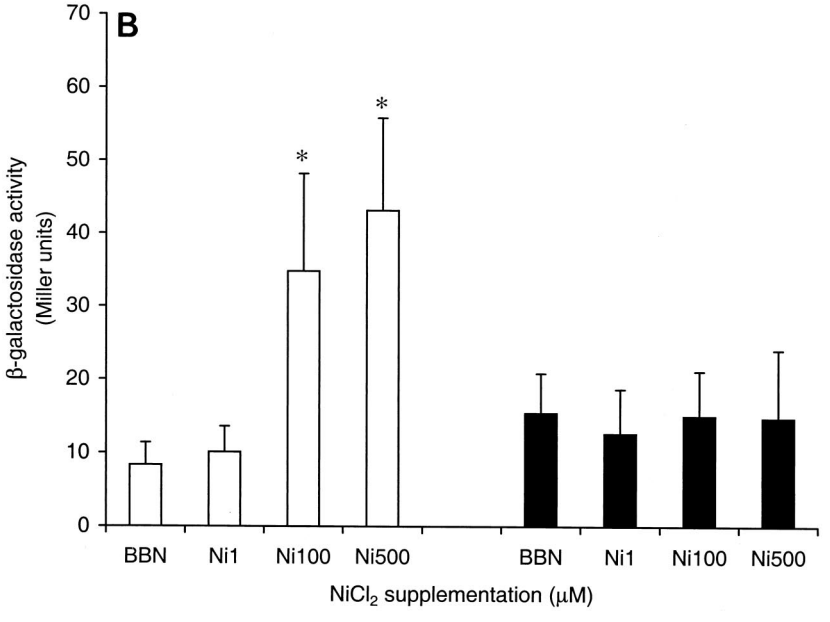


FIG. 3. Identification of a nickel-responsive operator sequence in the *H. pylori ureA* promoter. (A) Strategy used for identification of the operator sequence. The predicted palindromic structure is indicated by two converging arrows, and the palindrome structure and sequence are shown in the box next to the constructs. Promoter elements (−10 and −35) and the transcriptional start site (+1) are indicated. The boxed A′ indicates the truncated form of the *ureA* gene used for making the *ureA*::*lacZ* promoter fusions. (B) β-Galactosidase activities of the wild-type *ureA*::*lacZ* promoter fusion and a *ureA*(Δ−50/−70)::*lacZ* promoter fusion mutant in response to different nickel concentrations. White bars represent the wild-type *ureA*::*lacZ* promoter of *H. pylori* AV433; black bars represent the *ureA*(Δ−50/−70)::*lacZ* promoter of *H. pylori* 1061BJD3.10. Strains were grown in unsupplemented medium (BBN) or in BBN supplemented with $NiCl_2$ to final concentrations of 1 μM (Ni1), 100 μM (Ni100), and 500 μM (Ni500). Results shown are the averages of three independent experiments; error bars denote standard deviations. Asterisks indicate a significant increase in β-galactosidase activity compared to that of the wild-type promoter in unsupplemented medium (the *P* value was <0.05, as determined by Student's *t* test).

Removal of the palindromic sequence did not affect the transcription of the *ureA* promoter in unsupplemented growth medium (Fig. 3B); however, while the wild-type *ureA*::*lacZ* fusion was significantly induced in medium supplemented with 100 μM $NiCl_2$ or higher (Fig. 3B), the deletion mutants did not show any induction at increasing $NiCl_2$ concentrations [e.g., the *ureA*(Δ−50/−70) mutant] (Fig. 3B). The β-galactosidase activities of the *ureA*(Δ−50/−90) and *ureA*(Δ−50/−112) mutants did not differ significantly from that of the *ureA*(Δ−50/−70) mutant under all the tested medium conditions (data not shown).

DISCUSSION

Urease enzyme activity plays an essential role in gastric colonization by *H. pylori*, since *H. pylori* mutants devoid of urease activity were unable to colonize the gastric mucosa in animal models (13, 14, 37) even when the gastric pH was neutralized by the administration of proton pump inhibitors (14). *H. pylori* produces very large amounts of urease, up to 10% of its total protein content (3). The expression and activity of *H. pylori* urease are upregulated by increased availability of the nickel cofactor, a novel type of transcriptional regulation for bacterial ureases (33). Here we have demonstrated that this nickel-responsive induction is mediated via the HP1338 protein, a NikR ortholog.

NikR was originally identified in *E. coli* as the nickel-responsive repressor of the nickel uptake operon *nikABCDE* (9) and is homologous to members of the family of Fur regulatory proteins, which are involved mostly in metal-responsive repression of metal acquisition and oxidative stress defense (16). Biochemical characterization of the *E. coli* NikR protein indicated that it is a member of the ribbon-helix-helix group of regulatory proteins (6) and that, when complexed with nickel, it binds to a 5′-GTATGA-N_{16}-TCATAC-3′ inverted repeat sequence in the *E. coli nikA* promoter (6, 7). Both *H. pylori* genome sequences (2, 32) contain only one sequence resembling this *E. coli* NikR-binding sequence, which is located directly upstream of the *H. pylori nikR* gene, indicating possible autoregulation of *nikR* expression (7).

The urease operon contains two promoters, which are located upstream of the *ureA* and *ureI* genes (1, 33), leading to the differential expression of the subunit and accessory proteins. Nickel supplementation induces transcription only from the *ureA* promoter (33), and this induction is dependent both on the NikR protein (Fig. 2) and on the presence of a 19-bp palindromic sequence in the *ureA* promoter (Fig. 3). This palindrome closely resembles the iron-responsive regulator Fur in terms of the structure and binding sequence (16). It would be of interest to test the *ureA*::*lacZ* promoter variants used in this study in a *nikR* mutant of *H. pylori* strain 1061; unfortunately, however, we have been unsuccessful in mutating *nikR* in this strain (data not shown). It is also unfortunate that the use of the pBW-based *lacZ* reporter gene system is currently successful only with *H. pylori* strain 1061, despite attempts to use it with *H. pylori* strain 26695 (4, 11).

The role of *H. pylori* NikR in urease regulation is opposite the role of *E. coli* NikR in nickel uptake, since *H. pylori* NikR induces the transcription of urease genes while *E. coli* NikR represses the transcription of nickel uptake genes. This difference may be connected to the location of the palindrome, which is located upstream of the −35 and −10 promoter sequences in the *ureA* gene. We hypothesize that in unsupplemented medium, the *ureA* promoter is not completely accessible to RNA polymerase, leading to normal levels of expression of urease. Supplementation of growth medium with higher concentrations of $NiCl_2$ may lead to increased cytoplasmic availability of nickel, allowing the formation of a NikR-nickel complex which can subsequently bind to the palindrome in the *ureA* upstream region. Binding of NikR to the palindrome may make the *ureA* promoter more accessible to RNA polymerase, leading to increased levels of urease gene transcription.

The *nikR* mutant strain showed some increase in urease activity when the medium was supplemented with 1 μM $NiCl_2$, to levels similar to those seen in the wild-type strain (Fig. 2B). This increase, however, was not accompanied by a significant increase in the expression of the urease subunits UreA and UreB (Fig. 2A) (33) and is probably due to more efficient nickel activation of an inactive urease apoenzyme already present. As suggested previously (33), these findings indicate that rather than the amount of urease protein, the amount of the cofactor nickel is a limiting factor for the urease activity of *H. pylori*. Increased availability of nickel at a low pH, combined with increased influx of urea via the acid-activated UreI protein (27, 36), would enable quick activation of inactive apourease and the associated increase in resistance to acid shock.

In conclusion, the NikR protein of *H. pylori* induces the transcription, expression, and activity of the essential virulence factor urease in response to nickel. Future studies should focus on the specificity of binding of NikR to the urease promoter as well as the possible roles of NikR in the regulation of nickel uptake, storage, or efflux. The nickel-responsive induction of urease via NikR may also play a role in other urease-producing bacteria and may allow the development of new or improved strategies to prevent or control infection with urease-positive bacteria.

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REFERENCES

1. **Akada, J. K., M. Shirai, H. Takeuchi, M. Tsuda, and, T. Nakazawa.** 2000. Identification of the urease operon in *Helicobacter pylori* and its control by mRNA decay in response to pH. Mol. Microbiol. **36:**1071–1084.
2. **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.
3. **Bauerfeind, P., R. Garner, B. E. Dunn, and H. L. Mobley.** 1997. Synthesis and activity of *Helicobacter pylori* urease and catalase at low pH. Gut **40:**25–30.
4. **Bijlsma, J. J. E., C. M. J. E. Vandenbroucke-Grauls, S. H. Phadnis, and J. G. Kusters.** 1999. Identification of virulence genes of *Helicobacter pylori* by random insertion mutagenesis. Infect. Immun. **67:**2433–2440.
5. **Burne, R. A., and Y. M. Chen.** 2000. Bacterial ureases in infectious diseases. Microbes Infect. **2:**533–542.

6. **Chivers, P. T., and R. T. Sauer.** 1999. NikR is a ribbon-helix-helix DNA-binding protein. Protein Sci. **8:**2494–2500.
7. **Chivers, P. T., and R. T. Sauer.** 2000. Regulation of high-affinity nickel uptake in bacteria: Ni^{2+}-dependent interaction of NikR with wild-type and mutant operator sites. J. Biol. Chem. **275:**19735–19741.
8. **Clyne, M., A. Labigne, and B. Drumm.** 1995. *Helicobacter pylori* requires an acidic environment to survive in the presence of urea. Infect. Immun. **63:** 1669–1673.
9. **De Pina, K., V. Desjardin, M. A. Mandrand-Berthelot, G. Giordano, and L. F. Wu.** 1999. Isolation and characterization of the *nikR* gene encoding a nickel-responsive regulator in *Escherichia coli*. J. Bacteriol. **181:**670–674.
10. **de Reuse, H., and S. Skouloubris.** 2001. Nitrogen metabolism, p. 125–133. *In* H. L. T. Mobley, G. L. Mendz, and S. L. Hazell (ed.), *Helicobacter pylori*: physiology and genetics. ASM Press, Washington, D.C.
11. **de Vries, N., E. J. Kuipers, N. E. Kramer, A. H. M. van Vliet, J. J. E. Bijlsma, M. Kist, S. Bereswill, C. M. J. E. Vandenbroucke-Grauls, and, J. G. Kusters.** 2001. Identification of environmental stress-regulated genes in *Helicobacter pylori* by a *lacZ* reporter gene fusion system. Helicobacter **6:**300–309.
12. **Dunn, B. E., H. Cohen, and M. J. Blaser.** 1997. *Helicobacter pylori*. Clin. Microbiol. Rev. **10:**720–741.
13. **Eaton, K. A., C. L. Brooks, D. R. Morgan, and S. Krakowka.** 1991. Essential role of urease in pathogenesis of gastritis induced by *Helicobacter pylori* in gnotobiotic piglets. Infect. Immun. **59:**2470–2475.
14. **Eaton, K. A., and S. Krakowka.** 1994. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by *Helicobacter pylori*. Infect. Immun. **62:**3604–3607.
15. **Eitinger, T., and M. A. Mandrand-Berthelot.** 2000. Nickel transport systems in microorganisms. Arch. Microbiol. **173:**1–9.
16. **Escolar, L., J. Perez-Martin, and V. de Lorenzo.** 1999. Opening the iron box: transcriptional metalloregulation by the Fur protein. J. Bacteriol. **181:**6223–6229.
17. **Goodwin, A., D. Kersulyte, G. Sisson, S. J. Veldhuyzen van Zanten, D. E. Berg, and P. S. Hoffman.** 1998. Metronidazole resistance in *Helicobacter pylori* is due to null mutations in a gene (*rdxA*) that encodes an oxygen-insensitive NADPH nitroreductase. Mol. Microbiol. **28:**383–393.
18. **Grass, G., C. Grosse, and, D. Nies.** 2000. Regulation of the *cnr* cobalt and nickel resistance determinant of *Ralstonia* sp. strain CH34. J. Bacteriol. **182:**1390–1398.
19. **Hawtin, P. R., H. T. Delves, and D. G. Newell.** 1991. The demonstration of nickel in the urease of *Helicobacter pylori* by atomic absorption spectroscopy. FEMS Microbiol. Lett. **61:**51–54.
20. **Kuwahara, H., Y. Miyamoto, T. Akaike, T. Kubota, T. Sawa, S. Okamoto, and H. Madea.** 2000. *Helicobacter pylori* urease suppresses bactericidal activity of peroxynitrite via carbon dioxide production. Infect. Immun. **68:** 4378–4383.
21. **Makristathis, A., E. Rokita, A. Labigne, B. Willinger, M. L. Rotter, and A. M. Hirschl.** 1998. Highly significant role of *Helicobacter pylori* urease in phagocytosis and production of oxygen metabolites by human granulocytes. J. Infect. Dis. **177:**803–806.
22. **Makristathis, A., E. Rokita, E. Pasching, P. Apfalter, B. Willinger, M. L. Rotter, and, A. M. Hirschl.** 2001. Urease prevents adherence of *Helicobacter pylori* to Kato III gastric epithelial cells. J. Infect. Dis. **184:**439–445.
23. **Mobley, H. L. T.** 2001. Urease, p. 179–191. *In* H. L. T. Mobley, G. L. Mendz, and S. L. Hazell (ed.), *Helicobacter pylori*: Physiology and Genetics. ASM Press, Washington, D.C.
24. **Moeck, G. S., and J. W. Coulton.** 1998. TonB-dependent iron acquisition: mechanisms of siderophore-mediated active transport. Mol. Microbiol. **28:** 675–681.
25. **Nakamura, H., H. Yoshiyama, H. Takeuchi, T. Mizote, K. Okita, and T. Nakazawa.** 1998. Urease plays an important role in the chemotactic motility of *Helicobacter pylori* in a viscous environment. Infect. Immun. **66:**4832–4837.
26. **Rokita, E., A. Makristathis, E. Presterl, M. L. Rotter, and A. M. Hirschl.** 1998. *Helicobacter pylori* urease significantly reduces opsonization by human complement. J. Infect. Dis. **178:**1521–1525.
27. **Sachs, G., D. R. Scott, D. L. Weeks, M. Rektorscheck, and K. Melchers.** 2001. Regulation of urease for acid habitation, p. 277–283. *In* H. L. T. Mobley, G. L. Mendz, and S. L. Hazell (ed.), *Helicobacter pylori*: physiology and genetics. ASM Press, Washington, D.C.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **Shirai, M., R. Fujinaga, J. K. Akada, and T. Nakazawa.** 1999. Activation of *Helicobacter pylori ureA* promoter by a hybrid *Escherichia coli-H. pylori rpoD* gene in *E. coli*. Gene **239:**351–359.
30. **Smoot, D. T., H. L. Mobley, G. R. Chippendale, J. F. Lewison, and J. H. Resau.** 1990. *Helicobacter pylori* urease activity is toxic to human gastric epithelial cells. Infect. Immun. **58:**1992–1994.
31. **Tibazarwa, C., S. Wuertz, M. Mergeay, L. Wyns, and D. van Der Lelie.** 2000. Regulation of the *cnr* cobalt and nickel resistance determinant of *Ralstonia eutropha* (*Alcaligenes eutrophus*) CH34. J. Bacteriol. **182:**1399–1409.
32. **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, E. K. Hickey, D. E. Berg, J. D. Gocayne, T. R. Utterback, J. D. Peterson, J. M. Kelley, M. D. Cotton, J. M. Weidman, C. Fujii, C. Bowman, L. Watthey, E. Wallin, W. S. Hayes, M. Borodovsky, P. D. Karpk, H. O. Smith, C. M. Fraser, and J. C. Venter.** 1997. The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature **388:**539–547.
33. **van Vliet, A. H. M., E. J. Kuipers, B. Waidner, B. J. Davies, N. de Vries, C. W. Penn, C. M. J. E. Vandenbroucke-Grauls, M. Kist, S. Bereswill, and J. G. Kusters.** 2001. Nickel-responsive induction of urease expression in *Helicobacter pylori* is mediated at the transcriptional level. Infect. Immun. **69:**4891–4897.
34. **van Vliet, A. H. M., K. G. Wooldridge, and J. M. Ketley.** 1998. Iron-responsive gene regulation in a *Campylobacter jejuni fur* mutant. J. Bacteriol. **180:** 5291–5298.
35. **Weatherburn, M. W.** 1967. Phenol-hypochlorite reaction for the determination of ammonia. Anal. Chem. **39:**971–974.
36. **Weeks, D. L., S. Eskandari, D. R. Scott, and, G. Sachs.** 2000. A H^+-gated urea channel: the link between *Helicobacter pylori* urease and gastric colonization. Science **287:**482–485.
37. **Wirth, H. P., M. H. Beins, M. Yang, K. T. Tham, and M. J. Blaser.** 1998. Experimental infection of mongolian gerbils with wild-type and mutant *Helicobacter pylori* strains. Infect. Immun. **66:**4856–4866.
38. **Wren, B. W., J. Henderson, and J. M. Ketley.** 1994. A PCR-based strategy for the rapid construction of defined bacterial deletion mutants. BioTechniques **16:**994–996.

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