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The Nickel-Responsive Regulator NikR Controls Activation and Repression of Gene Transcription in *Helicobacter pylori*

Florian D. Ernst,[1] Ernst J. Kuipers,[1] Angela Heijens,[1] Roya Sarwari,[1] Jeroen Stoof,[1] Charles W. Penn,[2] Johannes G. Kusters,[1] and Arnoud H. M. van Vliet[1]*

Department of Gastroenterology and Hepatology, Erasmus MC-University Medical Center, Rotterdam, The Netherlands,[1] and School of Biosciences, University of Birmingham, Edgbaston, Birmingham, United Kingdom[2]

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The NikR protein is a nickel-dependent regulatory protein which is a member of the ribbon-helix-helix family of transcriptional regulators. The gastric pathogen *Helicobacter pylori* expresses a NikR ortholog, which was previously shown to mediate regulation of metal metabolism and urease expression, but the mechanism governing the diverse regulatory effects had not been described until now. In this study it is demonstrated that NikR can regulate *H. pylori* nickel metabolism by directly controlling transcriptional repression of NixA-mediated nickel uptake and transcriptional induction of urease expression. Mutation of the nickel uptake gene *nixA* in an *H. pylori* 26695 *nikR* mutant restored the ability to grow in Brucella media supplemented with 200 μM $NiCl_2$ but did not restore nickel-dependent induction of urease expression. Nickel-dependent binding of NikR to the promoter of the *nixA* gene resulted in nickel-repressed transcription, whereas nickel-dependent binding of NikR to the promoter of the *ureA* gene resulted in nickel-induced transcription. Subsequent analysis of NikR binding to the *nixA* and *ureA* promoters showed that the regulatory effect was dependent on the location of the NikR-recognized binding sequence. NikR recognized the region from −13 to +21 of the *nixA* promoter, encompassing the +1 and −10 region, and this binding resulted in repression of *nixA* transcription. In contrast, NikR bound to the region from −56 to −91 upstream of the *ureA* promoter, resulting in induction of urease transcription. In conclusion, the NikR protein is able to function both as a repressor and as an activator of gene transcription, depending on the position of the binding site.

The human gastric pathogen *Helicobacter pylori* colonizes the mucus layer covering the gastric epithelium. To colonize its acidic niche, *H. pylori* requires the activity of the nickel-containing urease and hydrogenase enzymes (21, 30), and thus it requires efficient acquisition of nickel from the environment. The main route for nickel uptake in *H. pylori* is via the NixA protein, which is a monomeric, high-affinity nickel transporter located in the cytoplasmic membrane (4, 24, 25, 27, 47). Expression of NixA is also required for efficient colonization of the gastric mucosa (29). Hence, the uptake and metabolism of nickel are of critical importance to *H. pylori*. When cytoplasmic nickel availability is insufficient, the urease and hydrogenase systems cannot be fully activated (39). This will impair survival of acid shocks, growth at acidic pHs, and colonization of the gastric mucosa (3, 10). However, high concentrations of nickel are also detrimental to the cell (28, 42). Nickel metabolism thus requires tight control to maintain cytoplasmic nickel concentrations within tolerable levels, by regulation of uptake, efflux, usage, and storage (28). Adaptation to such changes in the conditions inside or outside the bacterial cytoplasm is often achieved through transcriptional regulation of effector genes.

The nickel-responsive regulatory protein NikR is a member of the ribbon-helix-helix (RHH) family of DNA binding proteins (12). The NikR protein consists of two different domains: an N-terminal DNA-binding domain homologous to the Arc/CopG/MetJ/Mnt family of RHH regulators and a C-terminal domain that is required for binding of nickel and for tetramerization (8, 11–14, 35, 46). NikR was first identified in *Escherichia coli*, where it functions as a transcriptional repressor of the Nik nickel uptake system (20). NikR mediates its repressor function via nickel-dependent binding to a palindromic sequence in the promoter region of the *nik* operon (12, 14). The net result of this regulation is expression of the Nik system only when nickel is scarce in the cell (20).

NikR orthologs have been identified in other gram-negative bacteria, including *H. pylori* (15, 42). In *H. pylori*, NikR mediates nickel- and acid-responsive gene regulation (10, 15, 40–42) and is predicted to affect different pathways involved in metal metabolism (15, 39). NikR has been suggested to function as the main nickel-responsive regulatory system in *H. pylori*, since absence of NikR results both in reduced growth at higher environmental nickel concentrations and in the absence of nickel- and acid-responsive induction of urease expression (10, 15, 40, 42). However, these functions of NikR have been demonstrated mostly by using *H. pylori* mutant strains (10, 15, 40, 42), while evidence of direct regulation by NikR was not presented.

Here it is demonstrated that *H. pylori* NikR binds directly to specific sequences in the *nixA* and *ureA* promoters in a nickel-dependent fashion. This nickel-dependent binding of NikR to the *nixA* and *ureA* promoters results in repression and induction of transcription, respectively. The sequences recognized by *H. pylori* NikR are significantly different from the consensus sequence proposed for recognition by *E. coli* NikR. Based on

* Corresponding author. Mailing address: Department of Gastroenterology and Hepatology, Erasmus MC-University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands. Phone: 31-10-463-5944. Fax: 31-10-463-2793. E-mail: a.h.m.vanvliet@erasmusmc.nl.

TABLE 1. Oligonucleotide primers used in this study

Primer	Sequence (5′→3′)
NixA-F2-mut	GCTGTGAAATTGTGGTTTCC
NixA-R2-mut	CAAATAAGCCCACCAAGTAA
NixA-F1	GATCGCTTGGGCTAAAGAAC
NixA-R1-T7[a]	ctaatacgactcactatagggagaCGATTTCACTAGCGGTATCA
UreI-F2	AAGCACTGCGGTGATGAACT
UreI-R2-T7[a]	ctaatacgactcactatagggagaACCAATCGCCTTCAGTGATG
NIKRSK7-L1	ATGGTAGGTCTCAGCGCATGGATACACCCAATAAAGACGATT
NIKRSK7-R1	ATGGTAGGTCTCATATCATTCATTGTATTCAAAGCTAGACGCC
UreA-DFP-F	GTGGGCGTTTTATTGTTGAA
UreA-DFP-R-Dig[b]	ACTCTTTTGGGGTGAGTTTC
NixA-DFP-F	TGATGGCGATTTAGAAACCC
NixA-DFP-R-Dig[b]	GAGCAACGCTAAACCCAATG
Int-amiE-F1	ACTCATTGTGCGCTGTCAAG
Int-amiE-R1-Dig[b]	CCCGCATTCGCCCAAAGTAT

[a] Primer contains a 5′ extension with T7 promoter sequence (in lowercase letters) for the creation of an antisense RNA probe.
[b] Primer is digoxigenin labeled at the 5′ end.

these results, we hypothesize that the location of the operator sequence in the promoter region determines whether NikR represses or induces transcription in *H. pylori*.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. *H. pylori* strains used in this study were reference strain 26695 (38), its isogenic *nikR*::Km^r mutant (42), and an isogenic *nikR*::Km^r *nixA*::Cm^r mutant constructed for this study (see below). *H. pylori* was routinely cultured on Dent agar (41) at 37°C under microaerobic conditions (10% CO_2, 5% O_2, and 85% N_2). Broth cultures were grown in Brucella broth (Difco, Sparks, MD) supplemented with 3% newborn calf serum (Gibco Life Technologies) (BBN). Cultures were started at an optical density at 600 nm (OD_{600}) of 0.05 and shaken at 37°C and 40 rpm for a maximum of 24 h. BBN medium, as used in this study, contains ~0.2 μM of Ni^{2+} (6). $NiCl_2$ (Sigma) was used to supplement BBN medium to final concentrations of 20 and 200 μM. *E. coli* strains were grown aerobically at 37°C in Luria-Bertani medium (34). When appropriate, BBN and Luria-Bertani media were supplemented with ampicillin, kanamycin, or chloramphenicol to a final concentration of 100 μg/ml, 20 μg/ml, or 10 μg/ml, respectively.

Urease assay. The enzymatic activity of urease was determined in fresh *H. pylori* lysates by measuring ammonia production from hydrolysis of urea by using the Berthelot reaction as described previously (41). The concentration of ammonia in the samples was inferred from a standard NH_4Cl concentration curve. Enzyme activity was expressed as micromoles of substrate hydrolyzed per minute per milligram of protein. Protein concentrations were determined by the bicinchoninic acid method (Pierce) using bovine serum albumin as a standard.

Cloning, expression, and purification of *H. pylori* NikR. The *nikR* gene was amplified from *H. pylori* 26695 by using primers NIKRSK7-L1 and NIKRSK7-R1 (Table 1). The resulting fragment was digested with BsaI and ligated into BsaI-digested pASK-IBA7 (IBA, Gottingen, Germany) to create pASK-IBA7-NikR. The wild-type sequence of the *nikR* gene was confirmed by DNA sequencing. *H. pylori* NikR was expressed with an N-terminal Strep tag, which does not influence the DNA-binding activity of the *H. pylori* Fur protein (23, 43, 45) and therefore was not removed prior to use. The recombinant protein was purified as described in the manufacturer's instructions and designated Strep-NikR. The recombinant protein was more than 90% pure as determined by staining with Coomassie brilliant blue following electrophoresis on 12% sodium dodecyl sulfate-polyacrylamide gels (14). Purified protein was used directly for electrophoretic mobility shift and DNase I footprinting assays.

Construction of a *nikR nixA* double mutant. The region containing the *nixA* gene was amplified by using primers NixA-F2-mut and NixA-R2-mut (Table 1). The resulting 936-bp *nixA* PCR fragment was cloned into pGEM-T Easy (Promega). The *nixA* coding region of this plasmid was interrupted by insertion of the chloramphenicol resistance gene from pAV35 (44) into the unique BglII site, resulting in plasmid pAHJNcat. Plasmid DNA was prepared using Wizard spin columns (Promega) and was used for natural transformation (7) of the *H. pylori* 26695 *nikR* mutant. Correct replacement of the *nixA* gene by the interrupted copy was confirmed using PCR (not shown).

Purification and analysis of RNA. Total RNA was isolated from *H. pylori* 26695 and its isogenic *nikR* mutant using Trizol (Gibco Life Technologies) (41). Gel electrophoresis of RNA, transfer to positively charged nylon membranes (Roche), cross-linking, hybridization to digoxigenin (DIG)-labeled specific RNA probes, and detection of bound probe were performed as described previously (22, 41). Probes specific for *nixA* and *ureI* were synthesized by in vitro transcription using T7 RNA polymerase (Roche) and PCR products obtained with primers NixA-F1/NixA-R1-T7 and UreI-F2/UreI-R2-T7 (Table 1).

Electrophoretic mobility shift assays. The ureA-DFP-F and ureA-DFP-R-Dig primers (Table 1) were used to amplify a 430-bp fragment from plasmid pBJD3.3 (17), which contains the wild-type *ureA* promoter region from *H. pylori* strain 1061 (designated P*ureA*). These primers were also used to amplify a 390-bp fragment from plasmid pBJD3.9 (17, 42), where the region encompassing nucleotides −50 to −90 is deleted from the *ureA* promoter (17, 42) (designated P*ureA*-del). The 514-bp *nixA* promoter region fragment (designated P*nixA*) was amplified with primers NixA-DFP-F and NixA-DFP-R-Dig (Table 1). An internal fragment of the *H. pylori amiE* gene was amplified with primers Int-amiE-F1 and Int-amiE-R1-Dig (Table 1) and was used as a negative control. Electrophoretic mobility shift assays were performed using 18, 20, and 16 pM of P*ureA*-wt, P*ureA*-del, and P*nixA* promoter fragments, as well as with 43 pM of the negative control. DNA fragments were mixed with Strep-NikR protein to final concentrations of 0, 15, 30, 150, and 300 nM in binding buffer (consisting of 20 mM Tris [pH 7.6], 100 mM KCl, 3 mM $MgCl_2$, 0.1% Nonidet P-40, 5% glycerol, and 100 μM of $NiCl_2$) and incubated for 30 min at 37°C. Subsequently, samples were loaded onto nickel-containing 7% acrylamide gels (34). Gels were blotted onto a nylon membrane (Roche), followed by chemiluminescent DIG detection (41).

DNase I footprinting. DNase I footprinting was performed using 360, 400, and 320 pM of the P*ureA*, P*ureA*-del, and P*nixA* fragment, respectively. DNA fragments were incubated without or with 2.86 μM of Strep-NikR protein in the presence or absence of 100 μM $NiCl_2$ in binding buffer (10 mM HEPES [pH 7.6], 100 mM KCl, 3 mM $MgCl_2$, and 1.5 mM $CaCl_2$) for 30 min at 37°C. Subsequently the DNA was digested with 0.25 U DNase I (Promega) for 1 min, and the reaction was stopped as described previously (19). Fragments were separated on a 7% acrylamide–8 M urea sequencing gel (Bio-Rad) (34). Gels were blotted onto a positively charged nylon membrane (Roche), followed by chemiluminescent DIG detection (41).

RESULTS

Absence of NixA complements nickel sensitivity but does not restore urease regulation in an *H. pylori nikR* mutant. The main phenotypes of an *H. pylori* 26695 *nikR* mutant are reduced growth in BBN medium supplemented with $NiCl_2$ concentrations of >100 μM and the absence of nickel-responsive induction of urease expression (42). To examine the role of the NixA nickel transporter in these phenotypes, the *nixA* gene was interrupted in an *H. pylori* 26695 *nikR* mutant (42), thereby creating a *nikR nixA* double mutant. The growth of wild-type *H. pylori*, the *nikR* mutant, and the *nikR nixA* mutant did not differ in unsupplemented BBN medium or in BBN medium supplemented with 20 μM $NiCl_2$ (Fig. 1A). Consistent with our earlier data (42), growth of the *nikR* mutant was significantly decreased when BBN was supplemented with $NiCl_2$ to a final concentration of 200 μM (Fig. 1A). The decrease in growth of the *nikR* mutant after supplementation with $NiCl_2$ at concentrations of 40 μM or higher was accompanied by a significant decrease in viability (data not shown). In contrast, the *nikR nixA* double mutant grew to levels similar to those of the wild-type strain at the nonpermissive $NiCl_2$ concentration of 200 μM (Fig. 1A).

Mutation of *nixA* in the *nikR* mutant did not, however, restore nickel-responsive regulation of urease activity (Fig. 1B). Urease

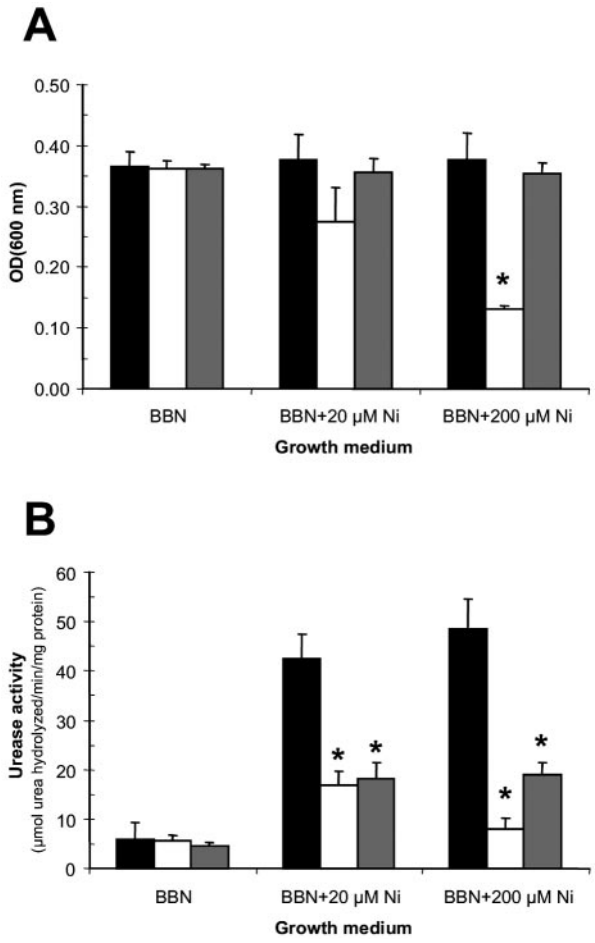

FIG. 1. Mutation of *nixA* complements nickel sensitivity of an *H. pylori nikR* mutant but does not restore nickel-dependent induction of urease activity. (A) Growth of wild-type *H. pylori* 26695 (black bars), the *nikR* mutant (white bars), and the *nikR nixA* double mutant (gray bars) in BBN medium supplemented with 0, 20, or 200 μM $NiCl_2$. Results are averages for three independent growth experiments after measurement of the OD_{600} 24 h after inoculation. (B) Urease activity measurements of wild-type *H. pylori* 26695 (black bars), the *nikR* mutant (white bars), and the *nikR nixA* double mutant (gray bars) grown in BBN medium supplemented with 0, 20, or 200 μM $NiCl_2$. Results are averages of three independent urease activity measurements. Error bar indicate standard deviations. Asterisks indicates a significant difference between the growth (A) or urease activity (B) of a mutant and that of the wild-type strain ($P < 0.05$ by the Mann-Whitney U test).

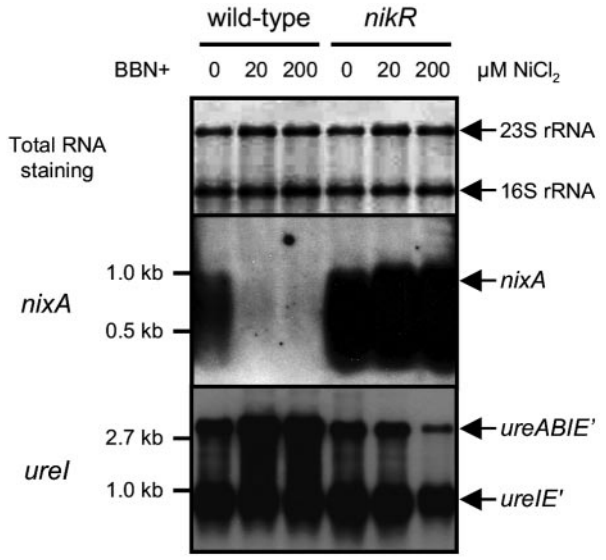

FIG. 2. Transcription of *nixA* is nickel and NikR repressed, whereas transcription of *ureA* is nickel and NikR induced, in *H. pylori*. Northern hybridization was performed on RNA from wild-type *H. pylori* 26695 and *nikR* mutant cells grown in BBN medium supplemented with 0, 20, or 200 μM $NiCl_2$. Staining of transferred RNA by methylene blue is included for comparison of RNA amounts (top panel). The positions of the predicted *nixA*, *ureABIE'*, and *ureIE'* transcripts (1, 41) are indicated on the right, whereas the probes used and relevant marker sizes are given on the left.

activity was relatively low in unsupplemented medium in all three strains (Fig. 1B). In medium supplemented with 20 or 200 μM $NiCl_2$, the wild-type strain displayed an increase in urease activity, which was not apparent in either the *nikR* mutant or the *nikR nixA* double mutant. Urease activity even decreased in the *nikR* mutant when it was grown in medium supplemented with 200 μM $NiCl_2$ (Fig. 1B), but this coincided with decreased growth of this strain under these conditions (Fig. 1A).

Transcription of the *nixA* and *ureA* genes is regulated by NikR. Northern hybridization with probes specific for the *nixA* and *ureI* genes was used to assess whether transcription of *nixA* and the urease operon is regulated by nickel and NikR. RNA was isolated from cultures grown in BBN medium supplemented with 0, 20, or 200 μM $NiCl_2$ (Fig. 1). The *nixA* probe hybridized to a transcript of approximately 1 kb in RNA isolated from wild-type *H. pylori* grown in unsupplemented medium but was not detected in RNA isolated from wild-type *H. pylori* grown in medium supplemented with 20 and 200 μM $NiCl_2$ (Fig. 2, center). In contrast, in the *nikR* mutant, transcription of the *nixA* gene was constitutively high and independent of $NiCl_2$ supplementation (Fig. 2, center). The size of the *nixA* mRNA is consistent with monocistronic transcription of *nixA*.

The *ureI*-specific probe hybridized to two fragments, which are predicted to represent the constitutively transcribed *ureIE'* mRNA (0.9 kb) and the nickel-responsive *ureABIE'* mRNA (3.4 kb) (1, 41). In wild-type *H. pylori* 26695, the amount of the 3.4-kb *ureABIE'* mRNA increased upon nickel supplementation compared to unsupplemented medium. In contrast, in the *nikR* mutant, nickel-responsive induction of the *ureABIE'* mRNA was abolished (Fig. 2, bottom). Taken together, these findings suggest that *H. pylori* NikR acts as a nickel-dependent repressor of *nixA* transcription and as a nickel-dependent activator of urease transcription.

NikR mediates repression of *nixA* transcription by nickel-dependent binding to the *nixA* promoter. A 514-bp fragment containing the *nixA* promoter region was amplified by PCR and incubated with Strep-NikR in the presence or absence of nickel (Fig. 3A). In the absence of nickel, addition of Strep-NikR protein did not result in an electrophoretic mobility shift (Fig. 3A). When nickel was present in the binding buffer, addition of the Strep-NikR protein resulted in an electrophoretic mobility shift (Fig. 3A). An internal fragment of the *H. pylori amiE* gene was used as a negative control and did not display any shift in the presence of nickel and Strep-NikR (Fig. 3A).

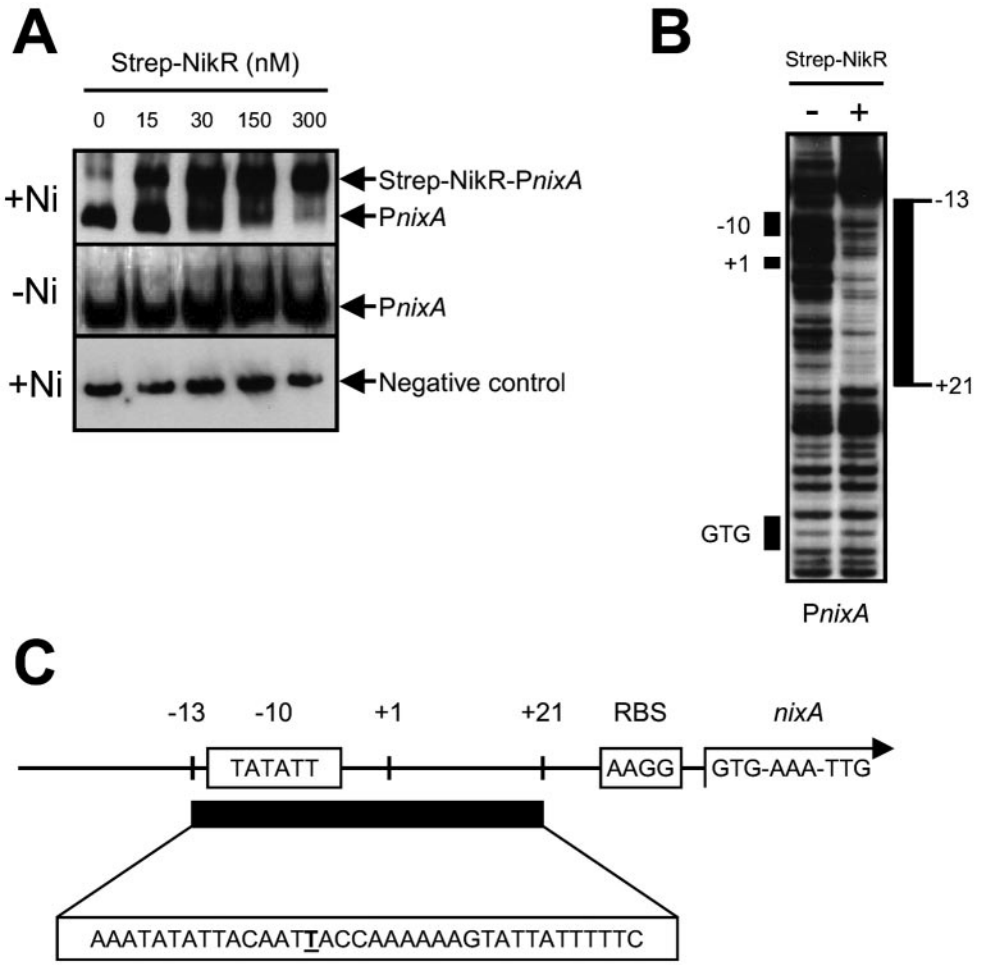

FIG. 3. NikR represses *nixA* transcription by nickel-dependent binding to a specific operator in the *H. pylori nixA* promoter region. (A) Electrophoretic mobility shift assays with recombinant *H. pylori* Strep-NikR protein and the *nixA* promoter (P*nixA*) in the presence (+Ni) and absence (−Ni) of $NiCl_2$. An internal fragment of the *amiE* gene was used as a negative control only in the presence of $NiCl_2$. The Strep-NikR-complexed *nixA* fragment is indicated as Strep-NikR-P*nixA*. Strep-NikR concentrations used are given above the lanes; DNA concentrations were 16 pM (P*nixA*) and 43 pM (negative control). (B) Identification of the NikR operator sequence in the *nixA* promoter by DNase I footprinting in the absence (−) and presence (+) of Strep-NikR protein, in the presence of nickel. The protected region is delineated by a black bar on the right, while the positions of the GTG start codon, the +1 transcriptional start site, and the −10 promoter region are indicated on the left. (C) Schematic representation of the *nixA* promoter region with the location and sequence of the NikR-binding site indicated, whereby −13 and +21 indicate the boundaries of the NikR-binding site. The *nixA* transcriptional start site (16) is underlined in the binding sequence.

The location of the binding sequence for NikR in the *nixA* promoter was identified using a DNase I footprinting assay (Fig. 3B). In the presence of nickel, Strep-NikR protein blocked DNase I degradation of a single sequence (AAA TATATTACAATTACCAAAAAAGTATTATTTTTC). Since the transcription start site of the *nixA* mRNA is the T residue 36 bp upstream of the GTG start codon (16), this sequence is located from −13 to +21 relative to this transcription start site (Fig. 3C). The protected region includes the transcriptional start site and the putative −10 promoter region (Fig. 3C). The region from −13 to + 21 was not protected against DNase I degradation by Strep-NikR in the absence of nickel (not shown).

NikR induces urease transcription by binding to an upstream operator sequence of *ureA*. A 430-bp fragment was amplified containing the wild-type *H. pylori ureA* promoter region (P*ureA*). In the presence of nickel, addition of Strep-NikR to P*ureA* resulted in an electrophoretic mobility shift, which was missing in the absence of nickel (Fig. 4A). Using a DNase I footprinting assay, it was demonstrated that in the presence of nickel, Strep-NikR protein consistently blocked DNase I degradation of a single binding sequence (CAAAGATATAACACT AATTCATTTTAAATAATAATT) located from −56 to −91 relative to the transcription start site (17) (Fig. 4B and C). The region bound by Strep-NikR was not protected against DNase I degradation in the absence of nickel or in the absence of NikR (Fig. 4B, left), consistent with the electrophoretic mobility shift assays (Fig. 4A).

It was previously suggested that the palindromic region present at positions −49 to −67 in the *ureA* promoter may be involved in the regulation of *ureA* transcription by NikR (17, 42). Using the *ureA* promoter deletion fragment P*ureA*-del, which lacks the sequence from positions −50 to −90, no mobility shift complex was observed in either the presence or the

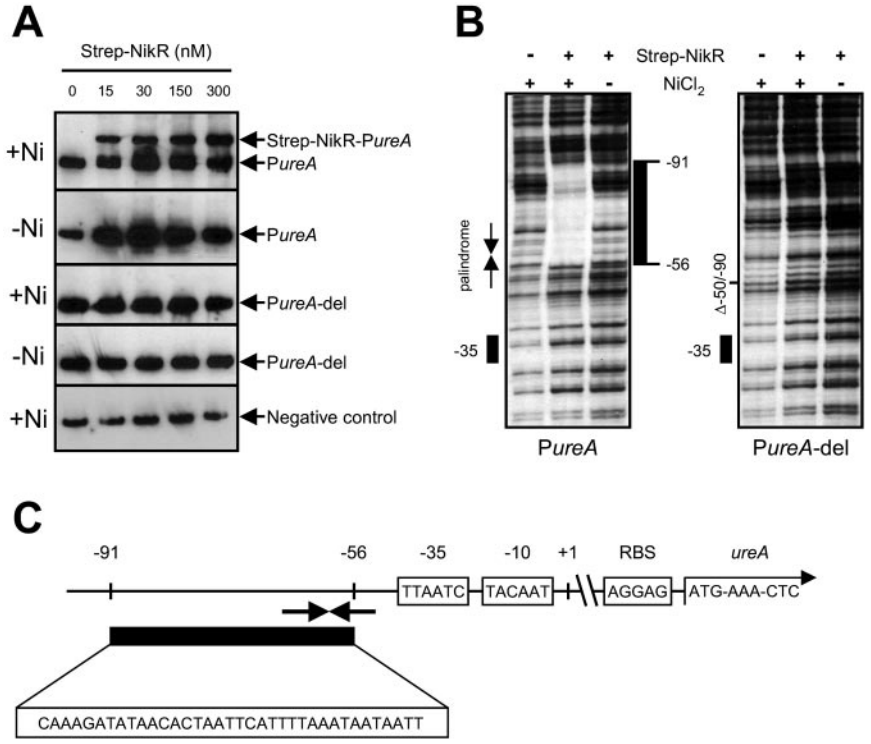

FIG. 4. NikR induces *ureA* transcription by nickel-dependent binding to a specific operator in the *H. pylori ureA* promoter region. (A) Electrophoretic mobility shift assays with recombinant *H. pylori* Strep-NikR protein and the *ureA* promoter (P*ureA*) and a −90/−50 deletion mutant (P*ureA*-del) in the presence (+Ni) and absence (−Ni) of $NiCl_2$. An internal fragment of the *amiE* gene was used as a negative control only in the presence of $NiCl_2$. The Strep-NikR-complexed *ureA* fragment is indicated as Strep-NikR-P*ureA*. Strep-NikR concentrations used are given above the lanes; DNA concentrations were 20 pM (P*ureA*), 22 pM (P*ureA*-del), and 43 pM (negative control). (B) DNase I footprinting assays with the P*ureA* promoter fragment (left panel) and the P*ureA*-del promoter fragment (right panel) in the absence (−) and presence (+) of Strep-NikR protein and in the absence or presence of nickel. The protected region is delineated by a black bar on the right of the P*ureA* panel. Convergent arrows indicate the position of the palindrome from −49 to −67 in the *ureA* promoter, while Δ−50/−90 indicates the position of the deletion in the *PureA*-del promoter fragment. The location of the −35 promoter sequence is also indicated. (C) Schematic representation of the *ureA* promoter region with the location and sequence of the NikR-binding site indicated, whereby −91 and −56 indicate the boundaries of the NikR-binding site. The two arrows represent the putative inverted repeat at positions −67 to −49.

absence of nickel (Fig. 4A). In addition, deletion of this region resulted in a complete lack of protection against DNase I digestion (Fig. 4B, right).

DISCUSSION

H. pylori expresses a NikR ortholog (HP1338), which is required for nickel-responsive induction of urease expression, nickel resistance, and acid-responsive gene regulation (10, 15, 40, 42). However, these effects were demonstrated mostly by using mutational studies, and thus the possibility remained that these phenotypes were secondary or indirect effects of the *nikR* mutation. In this study it is demonstrated that the *H. pylori* NikR protein is a DNA-binding protein that functions as an activator of urease expression and a repressor of NixA-mediated nickel uptake. The role of NikR in regulation of *nixA* expression is consistent with the nickel sensitivity of the *nikR* mutant (Fig. 1A), which is due to derepressed expression of the NixA nickel uptake system (Fig. 1A and 2). Next to its role in regulation of nickel uptake, the NikR protein also controls the usage of nickel by regulation of urease expression (Fig. 1B and 2). Both these regulatory phenomena are mediated at the transcriptional level (Fig. 2), by nickel-dependent binding of the NikR protein to specific sequences in the *nixA* and *ureA* promoters (Fig. 3 and 4).

Nickel-responsive regulation by NikR had been studied in depth only for *E. coli*, where NikR regulates the expression of the Nik nickel transporter system (14, 20). Once the intracellular concentration of nickel exceeds a certain threshold (13), *E. coli* NikR binds to a palindromic sequence (GTATGA-N_{16}-TCATAC) that overlaps with the −10 region of the *nikA* promoter. This is thought to effectively block access of RNA polymerase to the promoter and results in cessation of transcription (14, 20). This process allows the cell to maintain control of the intracellular nickel concentration. Similar forms of metal-responsive regulation have been described for other metals, such as the control of iron metabolism by Fur (2).

The *H. pylori* NikR binding sequences in the *nixA* and *ureA* promoters were identified using DNase I footprinting. The NikR binding sequence in the *nixA* promoter consists of a 36-bp sequence, which is located at positions −13 to +21 relative to the transcriptional start site. This region in the *nixA* promoter effectively overlaps with the −10 and +1 sequence, and this may prevent transcription upon binding of NikR. In contrast, the NikR-binding site in the *ureA* promoter is located upstream of the canonical σ^{80} promoter motifs (17, 36, 42), at

positions −56 to −91, and partially overlaps with the putative palindrome previously suggested as a possible binding sequence for NikR (42). Deletion of the region upstream of residue −50 in the *ureA* promoter was previously shown not to affect basal levels of urease expression (17) but prevented nickel-responsive induction of urease expression (42), and this is consistent with the position of the NikR-binding site in the *ureA* promoter as identified in this study. The deletion of the region from −50 to −90 indeed abolished binding of NikR (Fig. 4B), indicating the importance of this region in NikR binding and nickel-responsive regulation of urease transcription (17, 42). We hypothesize that binding of NikR to the *ureA* binding site allows RNA polymerase easier access to the *ureA* promoter, by a mechanism currently unknown.

The two binding sequences recognized by *H. pylori* NikR do not resemble the *E. coli* NikR binding sequence (GTATGA-N_{16}-TCATAC) (14) and thus exemplify the clear differences between the *E. coli* and *H. pylori* NikR systems. A single homolog of the *E. coli* sequence is present in the *H. pylori* genome, in the promoter of the *nikR* gene itself. Although binding of recombinant NikR to its own promoter was reported, this binding did not result in nickel-responsive regulation of the *nikR* gene (15). Taken together, these data suggest that the sequences recognized by *H. pylori* NikR differ significantly from the *E. coli* NikR consensus sequence. Alignments of the NikR-binding sites in the *nixA* and *ureA* promoters revealed that they have only relatively limited homology to each other (19/36 residues [Fig. 5A]). It is therefore not yet possible to define a consensus sequence for the *H. pylori* NikR-binding site.

The NikR protein is a member of the RHH family of regulatory proteins, which function as transcriptional regulators. Members of this family include the Mnt (9) and Arc (32) repressors of bacteriophage P22 as well as the activator AlgZ of *Pseudomonas aeruginosa* (5). Dual regulation of transcription is already known from the Arc regulatory protein, which upon binding to a target promoter can either slow down open-complex formation or accelerate promoter clearance and thereby can act both as a repressor and as an activator of transcription (37). The regulator AlgZ of *P. aeruginosa* is necessary for activation of *algD* (5) and recently was demonstrated to display autorepression (31).

Comparison of the positions of the NikR-binding sites in the *nixA* and *ureA* promoters with the regulatory responses observed suggests that the position of the binding site determines whether transcription of a NikR-controlled gene is nickel repressed or nickel induced (Fig. 5B). When the binding site overlaps with the promoter motifs, transcription is repressed, whereas binding of NikR upstream of the promoter motifs results in induction of transcription. A similar type of regulation was described recently for the ferric uptake regulator protein Fur in *Neisseria meningitidis*, where transcription of the *tbp2* gene is iron and Fur repressed by binding of Fur to a sequence overlapping the −10 and +1 sequence, and transcription of three other genes is induced in an iron-dependent manner by binding of Fur to sequences upstream of the promoter region (18). A similar type of regulation has also been reported for *Mycobacterium tuberculosis* IdeR (26, 33).

In conclusion, the NikR protein of *H. pylori* functions as a repressor or an activator of nickel-responsive transcription, depending on the position of its binding site. Binding is dependent on nickel, and this mechanism allows *H. pylori* NikR to control both the uptake and the usage of nickel, depending on intracellular nickel availability. Compared to the *E. coli* NikR system, which is currently known to regulate only nickel uptake, *H. pylori* NikR is a versatile regulatory protein that can control important aspects of nickel metabolism and virulence.

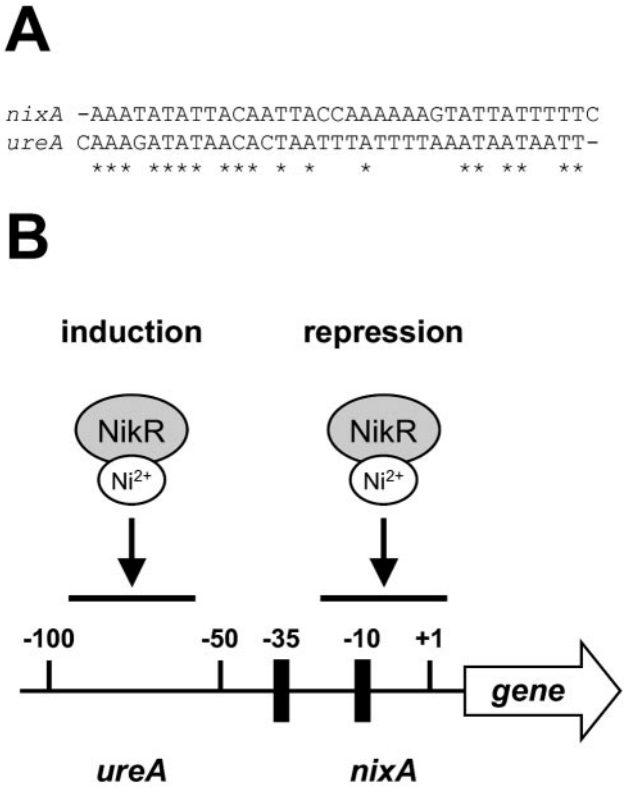

FIG. 5. Analysis and model of nickel-responsive gene regulation by *H. pylori* NikR. (A) Comparison of the *H. pylori* NikR-binding sites in the *nixA* and *ureA* promoters. The *H. pylori* NikR-binding sites in the *nixA* and *ureA* promoters were aligned using the Clone Manager 7 suite (Scientific and Educational Software, Cary, NC). Asterisks indicate identical residues. (B) Schematic overviews of NikR-mediated transcriptional regulation of *nixA* and *ureA* transcription in *H. pylori*. Binding of the NikR-nickel complex to sequences upstream of the canonical promoter (as in the *ureA* promoter) results in induction of transcription, whereas binding of the NikR-nickel complex to the canonical promoter results in repression of transcription (as in the *nixA* promoter).

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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. **Andrews, S. C., A. K. Robinson, and F. Rodriguez-Quinones.** 2003. Bacterial iron homeostasis. FEMS Microbiol. Rev. **27:**215–237.
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. **Bauerfeind, P., R. M. Garner, and L. T. Mobley.** 1996. Allelic exchange mutagenesis of *nixA* in *Helicobacter pylori* results in reduced nickel transport and urease activity. Infect. Immun. **64:**2877–2880.
5. **Baynham, P. J., A. L. Brown, L. L. Hall, and D. J. Wozniak.** 1999. *Pseudo-*

monas aeruginosa AlgZ, a ribbon-helix-helix DNA-binding protein, is essential for alginate synthesis and *algD* transcriptional activation. Mol. Microbiol. **33:**1069–1080.
6. **Bereswill, S., S. Greiner, A. H. van Vliet, B. Waidner, F. Fassbinder, E. Schiltz, J. G. Kusters, and M. Kist.** 2000. Regulation of ferritin-mediated cytoplasmic iron storage by the ferric uptake regulator homolog (Fur) of *Helicobacter pylori*. J. Bacteriol. **182:**5948–5953.
7. **Bijlsma, J. J., C. M. 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.
8. **Bloom, S. L., and D. B. Zamble.** 2004. Metal-selective DNA-binding response of *Escherichia coli* NikR. Biochemistry **43:**10029–10038.
9. **Burgering, M. J., R. Boelens, D. E. Gilbert, J. N. Breg, K. L. Knight, R. T. Sauer, and R. Kaptein.** 1994. Solution structure of dimeric Mnt repressor (1–76). Biochemistry **33:**15036–15045.
10. **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.
11. **Carrington, P. E., P. T. Chivers, F. Al-Mjeni, R. T. Sauer, and M. J. Maroney.** 2003. Nickel coordination is regulated by the DNA-bound state of NikR. Nat. Struct. Biol. **10:**126–130.
12. **Chivers, P. T., and R. T. Sauer.** 1999. NikR is a ribbon-helix-helix DNA-binding protein. Protein Sci. **8:**2494–2500.
13. **Chivers, P. T., and R. T. Sauer.** 2002. NikR repressor: high-affinity nickel binding to the C-terminal domain regulates binding to operator DNA. Chem. Biol. **9:**1141–1148.
14. **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.
15. **Contreras, M., J. M. Thiberge, M. A. Mandrand-Berthelot, and A. Labigne.** 2003. Characterization of the roles of NikR, a nickel-responsive pleiotropic autoregulator of *Helicobacter pylori*. Mol. Microbiol. **49:**947–963.
16. **Davies, B. J.** 2001. The expression, regulation and biological importance of the urease enzyme of *Helicobacter pylori.* Ph.D. thesis. University of Birmingham, Birmingham, United Kingdom.
17. **Davies, B. J., N. de Vries, S. G. Rijpkema, A. H. van Vliet, and C. W. Penn.** 2002. Transcriptional and mutational analysis of the *Helicobacter pylori* urease promoter. FEMS Microbiol. Lett. **213:**27–32.
18. **Delany, I., R. Rappuoli, and V. Scarlato.** 2004. Fur functions as an activator and as a repressor of putative virulence genes in *Neisseria meningitidis*. Mol. Microbiol. **52:**1081–1090.
19. **Delany, I., G. Spohn, R. Rappuoli, and V. Scarlato.** 2001. The Fur repressor controls transcription of iron-activated and -repressed genes in *Helicobacter pylori*. Mol. Microbiol. **42:**1297–1309.
20. **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.
21. **Eaton, K. A., J. V. Gilbert, E. A. Joyce, A. E. Wanken, T. Thevenot, P. Baker, A. Plaut, and A. Wright.** 2002. In vivo complementation of *ureB* restores the ability of *Helicobacter pylori* to colonize. Infect. Immun. **70:**771–778.
22. **Ernst, F. D., S. Bereswill, B. Waidner, J. Stoof, U. Mader, J. G. Kusters, E. J. Kuipers, M. Kist, A. H. M. van Vliet, and G. Homuth.** 2005. Transcriptional profiling of *Helicobacter pylori* Fur- and iron-regulated gene expression. Microbiology **151:**533–546.
23. **Ernst, F. D., G. Homuth, J. Stoof, U. Mader, B. Waidner, E. J. Kuipers, M. Kist, J. G. Kusters, S. Bereswill, and A. H. M. van Vliet.** 2005. Iron-responsive regulation of the *Helicobacter pylori* iron-cofactored superoxide dismutase SodB is mediated by Fur. J. Bacteriol. **187:**3687–3692.
24. **Fulkerson, J. F., Jr., R. M. Garner, and H. L. Mobley.** 1998. Conserved residues and motifs in the NixA protein of *Helicobacter pylori* are critical for the high affinity transport of nickel ions. J. Biol. Chem. **273:**235–241.
25. **Fulkerson, J. F., Jr., and H. L. Mobley.** 2000. Membrane topology of the NixA nickel transporter of *Helicobacter pylori*: two nickel transport-specific motifs within transmembrane helices II and III. J. Bacteriol. **182:** 1722–1730.
26. **Gold, B., G. M. Rodriguez, S. A. Marras, M. Pentecost, and I. Smith.** 2001. The *Mycobacterium tuberculosis* IdeR is a dual functional regulator that controls transcription of genes involved in iron acquisition, iron storage and survival in macrophages. Mol. Microbiol. **42:**851–865.
27. **Mobley, H. L., R. M. Garner, G. R. Chippendale, J. V. Gilbert, A. V. Kane, and A. G. Plaut.** 1999. Role of Hpn and NixA of *Helicobacter pylori* in susceptibility and resistance to bismuth and other metal ions. Helicobacter **4:**162–169.
28. **Mulrooney, S. B., and R. P. Hausinger.** 2003. Nickel uptake and utilization by microorganisms. FEMS Microbiol. Rev. **27:**239–261.
29. **Nolan, K. J., D. J. McGee, H. M. Mitchell, T. Kolesnikow, J. M. Harro, J. O'Rourke, J. E. Wilson, S. J. Danon, N. D. Moss, H. L. Mobley, and A. Lee.** 2002. In vivo behavior of a *Helicobacter pylori SS1 nixA* mutant with reduced urease activity. Infect. Immun. **70:**685–691.
30. **Olson, J. W., and R. J. Maier.** 2002. Molecular hydrogen as an energy source for *Helicobacter pylori*. Science **298:**1788–1790.
31. **Ramsey, D. M., P. J. Baynham, and D. J. Wozniak.** 2005. Binding of *Pseudomonas aeruginosa* AlgZ to sites upstream of the *algZ* promoter leads to repression of transcription. J. Bacteriol. **187:**4430–4443.
32. **Raumann, B. E., M. A. Rould, C. O. Pabo, and R. T. Sauer.** 1994. DNA recognition by β-sheets in the Arc repressor-operator crystal structure. Nature **367:**754–757.
33. **Rodriguez, G. M., and I. Smith.** 2003. Mechanisms of iron regulation in mycobacteria: role in physiology and virulence. Mol. Microbiol. **47:**1485–1494.
34. **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.
35. **Schreiter, E. R., M. D. Sintchak, Y. Guo, P. T. Chivers, R. T. Sauer, and C. L. Drennan.** 2003. Crystal structure of the nickel-responsive transcription factor NikR. Nat. Struct. Biol. **10:**794–799.
36. **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.
37. **Smith, T. L., and R. T. Sauer.** 1996. Dual regulation of open-complex formation and promoter clearance by Arc explains a novel repressor to activator switch. Proc. Natl. Acad. Sci. USA **93:**8868–8872.
38. **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.
39. **van Vliet, A. H. M., F. D. Ernst, and J. G. Kusters.** 2004. NikR-mediated regulation of *Helicobacter pylori* acid adaptation. Trends Microbiol. **12:**489–494.
40. **van Vliet, A. H. M., E. J. Kuipers, J. Stoof, S. W. Poppelaars, and J. G. Kusters.** 2004. Acid-responsive gene induction of ammonia-producing enzymes in *Helicobacter pylori* is mediated via a metal-responsive repressor cascade. Infect. Immun. **72:**766–773.
41. **van Vliet, A. H. M., E. J. Kuipers, B. Waidner, B. J. Davies, N. de Vries, C. W. Penn, C. M. 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.
42. **van Vliet, A. H. M., S. W. Poppelaars, B. J. Davies, J. Stoof, S. Bereswill, M. Kist, C. W. Penn, E. J. Kuipers, and J. G. Kusters.** 2002. NikR mediates nickel-responsive transcriptional induction of urease expression in *Helicobacter pylori*. Infect. Immun. **70:**2846–2852.
43. **van Vliet, A. H. M., J. Stoof, S. W. Poppelaars, S. Bereswill, G. Homuth, M. Kist, E. J. Kuipers, and J. G. Kusters.** 2003. Differential regulation of amidase- and formamidase-mediated ammonia production by the *Helicobacter pylori fur* repressor. J. Biol. Chem. **278:**9052–9057.
44. **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.
45. **Waidner, B., S. Greiner, S. Odenbreit, H. Kavermann, J. Velayudhan, F. Stahler, J. Guhl, E. Bisse, A. H. van Vliet, S. C. Andrews, J. G. Kusters, D. J. Kelly, R. Haas, M. Kist, and S. Bereswill.** 2002. Essential role of ferritin Pfrin *Helicobacter pylori* iron metabolism and gastric colonization. Infect. Immun. **70:**3923–3929.
46. **Wang, S. C., A. V. Dias, S. L. Bloom, and D. B. Zamble.** 2004. Selectivity of metal binding and metal-induced stability of *Escherichia coli* NikR. Biochemistry **43:**10018–10028.
47. **Wolfram, L., and P. Bauerfeind.** 2002. Conserved low-affinity nickel-binding amino acids are essential for the function of the nickel permease NixA of *Helicobacter pylori*. J. Bacteriol. **184:**1438–1443.

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