JOURNAL OF BACTERIOLOGY, Feb. 2006, p. 1245–1250 Vol. 188, No. 4
0021-9193/06/$08.00+0 doi:10.1128/JB.188.4.1245–1250.2006

Nickel Represses the Synthesis of the Nickel Permease NixA of *Helicobacter pylori*

Lutz Wolfram,[1] Elvira Haas,[2] and Peter Bauerfeind[1]*

Department of Internal Medicine, Division of Gastroenterology,[1] and Medical Policlinic,[2] University Hospital of Zurich, 8091 Zurich, Switzerland

Received 18 August 2005/Accepted 23 November 2005

Nickel acquisition is necessary for urease activity, a major virulence factor of the human gastric pathogen *Helicobacter pylori*. NixA was identified as a specific nickel uptake system in this organism. Addition of small amounts of nickel to media strongly stimulates urea hydrolysis. On the other hand, high nickel concentrations are deleterious to cell growth. As a possible protective reaction, nickel uptake seems to be reduced in *H. pylori* grown in nickel-rich media. These observations led to investigations of regulation of the expression of the nickel permease NixA. We found that increasing the nickel concentration in media reduced the amount of NixA. In order to address the question of whether this phenomenon was subject to transcriptional or translational regulation, we quantified *nixA* mRNA from *H. pylori* by real-time PCR. The amount of *nixA* mRNA was gradually reduced five- to sevenfold in a time- and concentration-dependent manner. Repression could be measured as soon as 5 min after nickel addition, and the maximum repression occurred after 20 to 30 min. The maximum repression was obtained with an external nickel concentration of 100 μM. The observed nickel repression of NixA was dependent on *nikR* encoding the nickel-responsive regulatory protein NikR. In conclusion, we demonstrated that synthesis of the NixA nickel permease of *H. pylori* shows nickel-responsive regulation mediated by NikR to maintain the balance between effective nickel acquisition and a toxic overload.

Helicobacter pylori is a gram-negative, microaerophilic human pathogen which colonizes the gastric mucosa of 50% of the world's population (17). *H. pylori* causes gastritis and is associated with the development of gastric and duodenal ulceration (6, 15, 34, 35), mucosa-associated lymphoid tissue lymphoma, and gastric cancer (7, 21). In order to withstand the acidic stomach environment, *H. pylori* synthesizes large amounts of the nickel-dependent enzyme urease (3, 27), an essential virulence and colonization factor for this organism (18, 19, 49). Urease catalysis leads to the formation of ammonia from the host's urea, which neutralizes the acidity in the vicinity of *H. pylori*. Nickel acquisition is indispensable for active urease production and survival of *H. pylori* in the human stomach. Since nickel is present at only trace levels in the human body (47) and low doses are obtained from the diet (2, 40), a specific uptake mechanism is needed. NixA has been identified as a high-affinity, low-capacity nickel permease in *H. pylori* (37). Another possibility for nickel entry, which is nonspecific, is CorA, the single magnesium uptake system present in *H. pylori* (42); however, nickel uptake via this system is rather limited under physiological conditions in terms of substrate affinities and the in situ concentration ratio (39, 42). Besides nickel, CorA can also transport cobalt and maybe ferrous iron (39). It has been shown that *H. pylori* is especially sensitive to cobalt (38), a metal which is not transported via NixA (T. Eitinger, personal communication).

The uptake, toxicity, resistance, and regulatory capacities of transition metals, especially the roles of iron (4, 5, 52) and nickel (38, 51), have been the subjects of several investigations of *H. pylori*. Since the role of nickel is ambivalent (it is toxic at higher concentrations but it is essential for urease activity), a delicate equilibrium of nickel uptake, storage, and incorporation into target enzymes is therefore necessary. The genome of *H. pylori* contains only a limited number of regulatory genes, which seemed to be consistent with the previous belief that *H. pylori* lives under rather constant environmental conditions in the gastric mucosa.

In general, nickel has been implicated in the regulation of several genes in bacteria, either inducing expression of nickel-dependent enzymes (29, 30, 50) and nickel resistance proteins (9, 25, 26) or repressing genes that encode nickel uptake proteins (11, 14). Analysis of nickel regulation at a molecular level has been achieved only with the NikR protein of *Escherichia coli*, which has recently been crystallized (45). NikR controls expression of the *nikABCDE* operon encoding the nickel-specific multiple-component ABC transporter in *E. coli*; *nikR* forms part of the operon as a sixth gene downstream (14). The homotetrameric NikR protein works as a direct nickel sensor and is a modular protein; the C-terminal two-thirds are necessary for tetramerization and high-affinity nickel binding (45), while the residues of the N-terminal part are involved in binding the target operator sequence (10), which was identified in the promoter region upstream of *nikA* (14). Binding of nickel-loaded NikR leads to repression of the *nik* operon and a reduction in nickel uptake (11).

An orthologue (HP1338/JHP1257) of the *E. coli nikR* gene was identified in the genome of *H. pylori* (1, 48). Recently, *nikR* has been implicated in nickel-dependent induction of urease synthesis, indicating that it is involved in nickel-dependent regulation (51). Addition of low levels of nickel (e.g., 1 to 5 μM) to culture media stimulates urease activity, whereas mod-

* Corresponding author. Mailing address: Department of Internal Medicine, Division of Gastroenterology, University Hospital of Zurich, Raemistr. 100, CH-8091 Zurich, Switzerland. Phone: 41-1-255 35 91. Fax: 41-1-255 45 03. E-mail: peter.bauerfeind@usz.ch.

erate concentrations inhibit bacterial growth partially and high concentrations prevent proliferation completely (the MICs are up to 2 mM, depending on the medium and strain) (51). Starting from our observations of retarded growth and reduced nickel uptake by *H. pylori* cells cultivated under excess-nickel conditions, we wanted to investigate whether the first step in nickel metabolism, nickel uptake, might be controlled by the external nickel concentration in the culture medium and whether it is dependent on NikR.

MATERIALS AND METHODS

Materials. Growth media and their components were obtained from Becton-Dickinson (brucella broth [BB], Bacto tryptone, Soytone, and Gelysate peptone), Merck (granulated yeast extract and sodium chloride), and Sigma (L-arginine, pyruvic acid [sodium salt], glucose, and cyclodextrin). Biochemicals were purchased from Boehringer Mannheim/Roche Molecular Biochemicals, Invitrogen, and QIAGEN. For detection of NixA-NixA antibody complexes in Western immunoblots, an alkaline phosphatase-coupled goat anti-rabbit antibody was used (Sigma). Nitrocellulose blotting membranes and nitrocellulose filters were obtained from Schleicher & Schuell.

Bacterial strains and growth determination. *H. pylori* wild-type strains ATCC 43504 and 26695 (= ATCC 700392) and *nikR* mutants of these strains were grown in BB, Wilkins-Chalgren (WC) broth, and Trypticase soy (TS) broth supplemented with 0.2% (wt/vol) cyclodextrins and with nickel at concentrations ranging from 0 to 1 mM. By using atomic absorbance spectroscopy the intrinsic nickel contents of brucella broth and Wilkins-Chalgren broth were determined to be 0.2 μM, whereas Trypticase soy broth was found to contain 1.0 μM nickel (Medizinisch-Chemisches Laboratorium, Basel, Switzerland). Plates and liquid cultures were incubated under water-saturated conditions at 37°C in jars (GasPack 100; Becton-Dickinson). Microaerophilic conditions were generated with CampyPak Plus (Becton-Dickinson) envelopes. The jars containing the liquid cultures were incubated in a G25 incubation shaker (New Brunswick Scientific) at 150 rpm. Growth was determined with a Jenway 6305 spectrophotometer by determining the optical density at 600 nm (OD_{600}). Overnight 1-ml liquid precultures (in BB) were used to inoculate the main cultures. At various times samples were withdrawn from the growing cultures and used for analysis of total RNA and/or measurement of the optical density. Care was taken that the time in air was as short as possible (usually 1 to 2 min).

Construction of a *nikR* mutant. *nikR* was amplified from the genome of *H. pylori* strain 26695 by PCR with appropriate primers (the forward primer introduced an EcoRI site upstream, and the reverse primer introduced a BamHI site downstream) and inserted into the vector pBluescript KS+ (Stratagene). After digestion with BclI (which led to internal loss of 81 bp in *nikR*), a filling-in step, and ligation with the KSF cassette (13), a plasmid with a nonfunctional *nikR* gene was obtained. This *nikR*-KSF construct was transformed into *H. pylori*.

Nickel uptake. Nickel transport measurements were carried out as described previously (53). In brief, cultures were inoculated with the same preculture using a dilution of 1:1,000 for the culture without added nickel and a dilution of 1:100 for the culture with 100 μM $NiCl_2$ to compensate for the growth retardation. This ensured that the cultures were still in the logarithmic growth phase after overnight incubation. *H. pylori* cells were then washed in transport buffer (50 mM Tris-HCl [pH 7.5], 10 mM $MgCl_2$) and concentrated 10-fold. For the assay the OD_{600} was adjusted to 0.3, and histidine (final concentration, 1 μg/ml) was added. Histidine is an effective nickel chelator and was used to reduce nonspecific uptake and binding in the assay. The suspension was incubated for 5 min at 37°C with shaking (G76 Gyrotory water bath shaker; speed 8; New Brunswick Scientific, Edison, NJ) prior to addition of $^{63}NiCl_2$ (24.7 TBq/mol) to a final concentration of 50 nM. After 0.25, 1, 2, and 5 min 400 μl of the suspension was passed through a nitrocellulose filter with a pore size of 0.2 μm. After this the filter was washed twice with 4 ml of transport buffer. The radioactivity was determined by liquid scintillation counting with a Packard 1900 TR counter. Statistical analysis was carried out using the mixed-effect linear model described previously (43).

Western blot analysis. Membrane proteins (30 μg) of *H. pylori* strains after 2 days of growth in liquid culture were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10% polyacrylamide) and electroblotted (semidry procedure; 10 V, 10 min, and 2 A with a Power-Pac 200 power supply; Bio-Rad) on nitrocellulose membranes as previously described (53). NixA was detected by antibodies to NixA raised in rabbits (a gift from H. L. T. Mobley, University of Maryland, Baltimore).

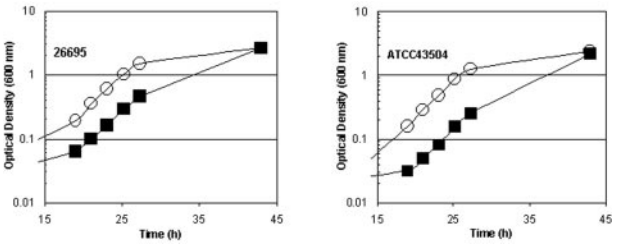

FIG. 1. Influence of an elevated nickel concentration (100 μM) on the growth of *H. pylori*. Cultures of strains ATCC 43504 and 26695 with and without added nickel were inoculated (1/1,000) with the same precultures. At each time the optical density at 600 nm was determined. ○, cultures without added nickel; ■, cultures to which 100 μM $NiCl_2$ was added.

RNA isolation and reverse transcription. Total RNA was extracted from *H. pylori* treated with lysozyme (0.4 mg/ml for 5 min at 37°C) using the silica-based RNeasy method (QIAGEN, Hilden, Germany), including an additional clean-up step to remove residual DNA as described in the manual of the manufacturer. The purity of RNA was controlled by reverse transcription reactions (PCR with nontranscribed RNA). RNA was reverse transcribed with an Omniscript RT kit (QIAGEN, Hilden, Germany).

Real-time quantitative PCR. Real-time quantitative PCR was used to determine the expression of genes encoding the NixA nickel permease (*nixA*; HP1077 locus according to Tomb et al. [48]) and the housekeeper glyceraldehyde-3-phosphate dehydrogenase (*gap*; HP0921 locus according to Tomb et al. [48]). For amplification of a *gap*-specific cDNA fragment 5′-TGCTAACGACCATTCATAG-3′ was used as the forward primer and 5′-GATTGGGTAACACTTTATGC-3′ was used as the reverse primer; and for amplification of a *nixA*-specific fragment 5′-ATGCGGCGGCGTCTATGG-3′ was used as the forward primer and 5′-TTGCCTTGT TGGGTGAGCTTTC-3′ was used as the reverse primer. A two-step PCR was performed with an iQ SYBR Supermix PCR kit (Bio-Rad) as follows: activation of the hot-start *Taq* polymerase for 3 min at 95°C, followed by 40 cycles of denaturation at 95°C for 15 s (step 1) and annealing and extension at 60°C for 1 min (step 2). Fluorescence was detected at the end of each extension step. The identities and specificities of amplicons were confirmed by agarose gel electrophoresis, melting curve analysis, and sequencing (Microsynth, Balgach, Switzerland). Gene expression was calculated using the $2^{-\Delta CT}$ method (32).

RESULTS

Excessive nickel retards growth of *H. pylori*. Under standard growth conditions without addition of nickel, *H. pylori* grew in 36 to 40 h to the maximal density (OD_{600}, 1.5 to 2.3) when it was inoculated at a 1:1,000 dilution from a fresh overnight preculture in BB. If 100 μM $NiCl_2$ was added to standard BB, which intrinsically contains 0.2 μM Ni^{2+}, the growth of the nickel-supplemented culture was retarded for 5 h, as shown for strains ATCC43504 and 26695 in Fig. 1. Cultures with and without added nickel grew with the same doubling time (about 2.5 to 3 h) and to the same final density (Fig. 1); this indicated that the bacteria were fit in the presence of nickel and just needed to adapt to a suddenly higher concentration. A similar growth retardation effect with excess nickel was seen in WC broth (intrinsically containing 0.2 μM Ni^{2+}) and in TS broth, which had an intrinsic nickel content of 1.0 μM Ni^{2+}.

***H. pylori* grown with excess nickel shows reduced nickel transport.** We assumed that excess nickel initiated some regulatory response for nickel uptake besides other possible events in the observed growth retardation. Therefore, we examined whether cells grown in the presence of high nickel concentrations had reduced nickel transport. The transport

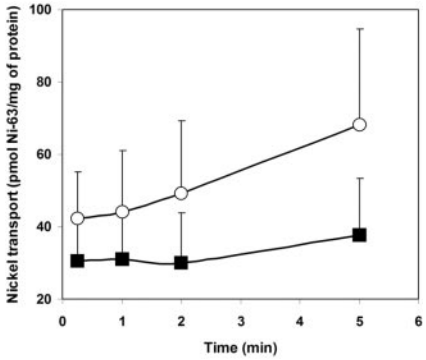

FIG. 2. Influence of nickel in growth media on nickel transport in *H. pylori*. Cells of strain 26695 were harvested in the logarithmic growth phase, and the OD_{600} was adjusted to 0.3 with transport buffer which contained 10 mM $MgCl_2$ (to suppress nickel uptake via CorA). The assay was started by addition of 50 nM $^{63}NiCl_2$. The difference in nickel uptake is statistically significant ($P = 0.0002$). ○, uptake by cells grown without added nickel; ■, uptake by cells grown in the presence of 100 μM $NiCl_2$.

buffer contained 10 mM $MgCl_2$ and only 50 nM $^{63}NiCl_2$ to suppress nickel uptake via CorA and promote nickel transport via specific systems. Figure 2 shows that transport in cells that grew in the presence of 100 μM $NiCl_2$ was reduced; a statistical analysis of the data from seven experiments revealed that there was a significant difference ($P = 0.0002$) in the nickel transport rates between bacteria grown with added nickel in BB and bacteria grown without added nickel in BB.

Increasing the nickel concentration in media reduces expression of NixA in *H. pylori*. We investigated the amount of NixA protein in *H. pylori* membranes with a NixA-specific antibody. The results for *H. pylori* strains ATCC 43504 and 26695 grown for 2 days are shown in Fig. 3; a reduction in the NixA signal to 20 to 50% (strain dependent) of the level found in membranes of bacteria grown in unsupplemented media was observed with 5 μM $NiCl_2$, whereas at higher concentrations the NixA signal gradually became weaker, until only traces of NixA were detected in the Western blot, starting with 50 and 100 μM for strains 26695 and ATCC 43504, respectively (Fig. 3). Still, NixA could be detected at low levels even with high nickel concentrations. This expression control of NixA could also be verified in membrane extracts of *H. pylori* grown in WC broth and TS broth (data not shown).

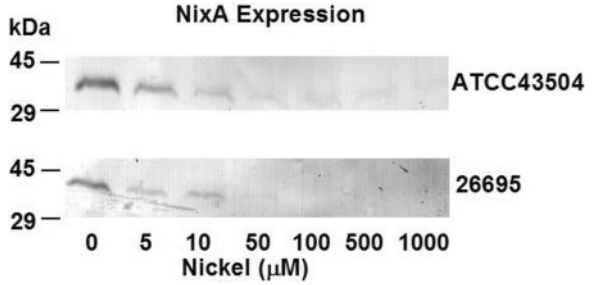

FIG. 3. Total amount of NixA in *H. pylori* in response to added nickel in the growth media. The numbers on the left indicate the molecular masses of marker proteins. The upper blot is a blot for strain ATCC 43504, and the lower blot is a blot for strain 26695. Each lane was loaded with 30 μg of protein.

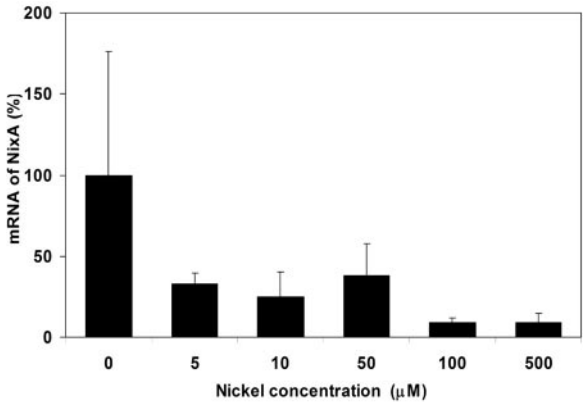

FIG. 4. Nickel-dependent repression of the *nixA* message. The bars indicate the percentages of the *nixA* message with different nickel concentrations in relation to the *nixA* message of cells grown without added nickel in BB.

Nickel represses NixA synthesis at the transcriptional level. In order to investigate whether the observed downregulation of NixA in membranes of *H. pylori* was regulated at the translational or transcriptional level, we isolated total RNA from *H. pylori* and examined whether the message of *nixA* was reduced in cells that grew in the presence of elevated nickel concentrations. As an internal standard the housekeeping gene *gap* encoding the glycerolaldehyde-3-phosphate dehydrogenase (HP0921 [48]) was used. We investigated the repression of *nixA* at $NiCl_2$ concentrations ranging from 0 to 500 μM (Fig. 4) and found that at 5 μM $NiCl_2$ the copy number of transcripts decreased by about one-half; at 100 μM $NiCl_2$ only 20% of the transcripts were detectable, and there was no further reduction at higher concentrations. Similar results were obtained with cells grown in TS broth (data not shown).

Repression of *nixA* is fast, efficient, and mediated by NikR. We then tried to determine how fast *H. pylori* downregulates the *nixA* transcript in the presence of nickel. In the first experiment we removed samples at 30, 60, 120, and 180 min after addition of nickel (final concentration, 100 μM). After 30 min complete downregulation was observed (to about 20% of the situation prior to nickel addition). In the subsequent experiments we removed samples for RNA isolation and quantification of *nixA* transcription after 5, 10, 20, 30, and 60 min (Fig. 5). It was evident that after 20 min the cells seemed to have finished adapting transcription of *nixA* to the new, lower level. When in parallel a culture was grown without nickel addition or when nickel was added to a culture of a *nikR* mutant, we did not observe a reduction in the *nixA* message at similar times. Accordingly, when we performed a Western blot analysis with membrane proteins and targeted NixA, a reduction in the amount of NixA in a *nikR* mutant was not observed, in contrast to the results obtained for the wild type (Fig. 6) after 2 days of growth.

DISCUSSION

The present work showed that externally supplied nickel negatively regulates the synthesis of the nickel permease NixA in *H. pylori* at the transcriptional level. With increasing nickel concentrations in the media, *nixA* mRNA is downregulated,

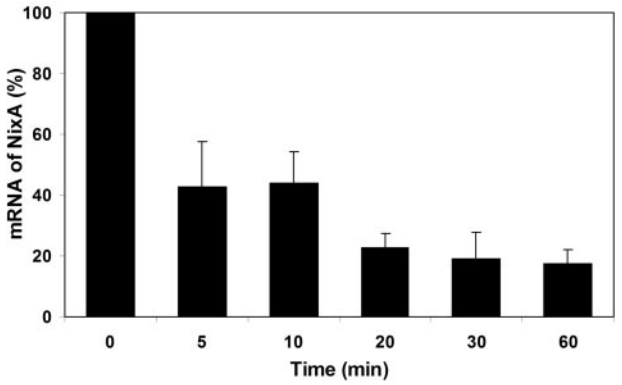

FIG. 5. Kinetics of *nixA* message reduction after nickel addition. The bars indicate the percentages of *nixA* message at different times after addition of nickel (100 μM) in relation to the *nixA* message before nickel was added.

and as a consequence, the level of NixA is reduced to basal levels. In this study nickel-dependent repression was observed with two different *H. pylori* strains (ATCC 43504 and 26695) and three different growth media (brucella broth, Wilkins-Chalgren broth, and Trypticase soy broth). The concentration of environmental nickel plays an important role in regulation of nickel uptake in *H. pylori*. The three media tested contain 0.2 μM nickel (brucella broth and Wilkins-Chalgren broth) and 1.0 μM nickel (Trypticase soy broth), and the bioavailabilities are different depending on the complexing capacity of the components. The presence of low nickel concentrations (e.g., 5 μM $NiCl_2$ in brucella broth) in the environment leads to a considerable decrease in transcription of *nixA*. The true concentration of nickel in the stomach or in the mucus of the stomach is unknown; it depends on the nickel content of the ingested nutrients and may vary widely during periods of fasting and food intake. The levels of daily dietary nickel intake fluctuate between 1 μmol (40) and 4 μmol (2), which should result in nickel concentrations in the range where nickel-dependent regulation was observed. NixA presumably should be able to ensure nickel uptake with minimal external concentrations. Assuming that the reduced synthesis of NixA is a protective measure against the toxic effect of an increased intracellular nickel content, it is noteworthy that downregulation of NixA starts at concentrations far below the MIC for cell growth (between 1 and 2 mM) (38).

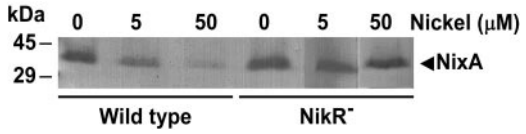

FIG. 6. NikR is responsible for the downregulation of NixA under excess-nickel conditions. In Western blots of membrane proteins from *H. pylori* NixA was the target protein. Each lane was loaded with 30 μg of protein. The numbers on the left indicate the molecular masses of the standards, and the arrowhead on the right indicates the position of the NixA signal. In the three left lanes membrane proteins of the wild type were loaded, and in the three right lanes membrane proteins of a *nikR* mutant were loaded; both strains were grown in the presence of three different nickel concentrations (0, 5, and 50 μM).

The *nixA* message of cells grown in the presence of excess nickel (100 μM nickel chloride or more) does not seem to be reduced to less than 15 to 20% of the message under standard conditions (no nickel added in brucella broth). When nickel was added to cells in the logarithmic phase of growth, repression was measurable only 5 min later, demonstrating the velocity, effectiveness, and sensitivity of the process. In this context it is surprising that the growth of *H. pylori* was retarded for several hours when the cells were directly inoculated into nickel-containing media. This could be explained by (i) the ratio of nickel ions to cell number, which was about 500 to 1,000 times higher; (ii) the fact that the inoculated cells were taken from a fresh overnight culture and not from an exponentially growing culture; and (iii) the fact that overall adaptation is needed when a few cells are added to fresh medium instead of addition of a small amount of a substance to actively growing cells. Another possibility is that about 75% of the population died, since the 5-h shift roughly equals two doubling times. It may be that both explanations are true for the observed growth retardation. In a *nikR* background nickel-dependent repression did not occur, which shows that NikR is the responsible regulatory protein. This is in agreement with the finding that NikR is a nickel-sensing pleiotropic regulator in *H. pylori* involved in acid adaptation (8, 50). The inability to repress NixA in a *nikR* mutant is accompanied by nickel sensitivity (51).

By comparing the transcriptomes of the wild type and a *nikR* mutant, both grown under excess-nickel conditions (250 μM nickel in brain heart infusion broth supplemented with 10% fetal calf serum), Contreras et al. (12) identified a whole regulon of NikR-controlled genes. Using macroarrays, they found that under excess-nickel conditions genes coding for nickel-consuming (*ureAB*) (27, 31), nickel-binding (*hpn* and *hpn*-like) (12, 22, 24, 38), and metal resistance (*copA2*) (23) proteins, as well as *nixA* (37), were activated, while genes for ferric ion uptake and storage (20), hydrogenase (33), motility (28, 36), stress responses (16, 46), and porin function (44) were repressed (12). The exact expression ratio for the wild-type and *nikR* mutant strains was not given, and the dot blot for confirmation of nickel-dependent activation of *nixA* expression is unimpressive. Since Contreras et al. (12) did not present a second line of evidence, it could be that in this case the nickel-dependent activation of *nixA* expression was found to be a significant but in fact was a false-positive result (41) The array-based finding of nickel-dependent activation of *nixA* expression does not explain the nickel sensitivity of the *nikR* mutant that Contreras et al. also observed at nickel chloride concentrations of 500 μM or higher (12). In their review van Vliet et al. (50) related NikR-dependent regulation to acid adaptation. Under acidic conditions the greater availability of nickel leads to the formation of nickel-NikR complexes. Nickel-loaded NikR increases its own expression and urease expression, but leads, in accordance with our results, to repression of NixA-mediated nickel transport and the iron regulator Fur. This allows NikR to control directly ammonia production by urease and nickel entry and to control indirectly the expression of iron metabolism, amidase-mediated ammonia production, and hydrogenase-based energy production via repression of *fur* (50). NikR is the key regulator for preventing damage caused by acid and/or nickel overload in *H. pylori* (50). Altogether, the increased nickel sensitivity of the *nikR* mutant supports our

experimental data for NikR-mediated repression of the nickel permease NixA under excess-nickel conditions. The fast, sensitive, and effective regulation by NixA might indicate that this protein has a significant role in nickel acquisition in *H. pylori* under the natural conditions found in the human stomach, where nickel concentrations are very low and may increase to bacteriotoxic levels during food intake.

ACKNOWLEDGMENTS

We thank Emerita Ammann for skilled technical assistance, Harry L. T. Mobley for providing the NixA-specific antibody, and R. Rappuoli for providing plasmid pKSF.

This work was supported by Swiss National Fund grant 32-66947.01.

REFERENCES

1. **Alm, R. A., and T. J. Trust.** 1999. Analysis of the genetic diversity of *Helicobacter pylori*: the tale of two genomes. J. Mol. Med. **77:**834–846.
2. **Barceloux, D. G.** 1999. Nickel. J. Toxicol. Clin. Toxicol. **37:**239–258.
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. **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.
5. **Bijlsma, J. J., B. Waidner, A. H. van Vliet, N. J. Hughes, S. Hag, S. Bereswill, D. J. Kelly, C. M. Vandenbroucke-Grauls, M. Kist, and J. G. Kusters.** 2002. The *Helicobacter pylori* homologue of the ferric uptake regulator is involved in acid resistance. Infect. Immun. **70:**606–611.
6. **Blaser, M. J.** 1990. *Helicobacter pylori* and the pathogenesis of gastroduodenal inflammation. J. Infect. Dis. **161:**626–633.
7. **Blaser, M. J., G. I. Perez-Perez, H. Kleanthous, T. L. Cover, R. M. Peek, P. H. Chyou, G. N. Stemmermann, and A. Nomura.** 1995. Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res **55:**2111–2115.
8. **Bury-Mone, S., J. M. Thiberge, M. Contreras, A. Maitournam, A. Labigne, and H. De Reuse.** 2004. Responsiveness to acidity via metal ion regulators mediates virulence in the gastric pathogen *Helicobacter pylori*. Mol. Microbiol. **53:**623–638.
9. **Cavet, J. S., W. Meng, M. A. Pennella, R. J. Appelhoff, D. P. Giedroc, and N. J. Robinson.** 2002. A nickel-cobalt-sensing ArsR-SmtB family repressor. Contributions of cytosol and effector binding sites to metal selectivity. J. Biol. Chem. **277:**38441–38448.
10. **Chivers, P. T., and R. T. Sauer.** 1999. NikR is a ribbon-helix-helix DNA-binding protein. Protein Sci. **8:**2494–2500.
11. **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.
12. **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.
13. **Copass, M., G. Grandi, and R. Rappuoli.** 1997. Introduction of unmarked mutations in the *Helicobacter pylori vacA* gene with a sucrose sensitivity marker. Infect. Immun. **65:**1949–1952.
14. **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.
15. **Dixon, M. F.** 1991. *Helicobacter pylori* and peptic ulceration: histopathological aspects. J. Gastroenterol. Hepatol. **6:**125–130.
16. **Donahue, J. P., D. A. Israel, V. J. Torres, A. S. Necheva, and G. G. Miller.** 2002. Inactivation of a *Helicobacter pylori* DNA methyltransferase alters *dnaK* operon expression following host-cell adherence. FEMS Microbiol. Lett. **208:**295–301.
17. **Dunn, B. E., H. Cohen, and M. J. Blaser.** 1997. *Helicobacter pylori*. Clin. Microbiol. Rev. **10:**720–741.
18. **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.
19. **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.
20. **Ernst, F. D., S. Bereswill, B. Waidner, J. Stoof, U. Mader, J. G. Kusters, E. J. Kuipers, M. Kist, A. H. van Vliet, and G. Homuth.** 2005. Transcriptional profiling of *Helicobacter pylori* Fur- and iron-regulated gene expression. Microbiology **151:**533–546.
21. **Forman, D., D. G. Newell, F. Fullerton, J. W. G. Yarnell, A. R. Stacey, N. Wald, and F. Sitas.** 1991. Association between *Helicobacter pylori* infection and risk of gastric cancer: evidence from a prospective study. Br. Med. J. **302:**1302–1305.
22. **Ge, R., R. M. Watt, X. Sun, J. A. Tanner, Q.-Y. He, J.-D. Huang, and H. Sun.** 2006. Expression and characterization of a histidine-rich protein, Hpn: potential for Ni^{2+} storage in *Helicobacter pylori*. Biochem. J. **393:**285–293.
23. **Ge, Z., and D. E. Taylor.** 1996. *Helicobacter pylori* genes *hpcopA* and *hpcopP* constitute a *cop* operon involved in copper export. FEMS Microbiol. Lett. **145:**181–188.
24. **Gilbert, J. V., J. Ramakrishna, J. F. W. Sunderman, A. Wright, and A. G. Plaut.** 1995. Protein Hpn: cloning and characterization of a histidine-rich metal-binding polypeptide in *Helicobacter pylori* and *Helicobacter mustelae*. Infect. Immun. **63:**2682–2688.
25. **Grass, G., B. Fan, B. P. Rosen, K. Lemke, H. G. Schlegel, and C. Rensing.** 2001. NreB from *Achromobacter xylosoxidans* 31A is a nickel-induced transporter conferring nickel resistance. J. Bacteriol. **183:**2803–2807.
26. **Grass, G., C. Grosse, and D. H. Nies.** 2000. Regulation of the *cnr* cobalt and nickel resistance determinant from *Ralstonia* sp. strain CH34. J. Bacteriol. **182:**1390–1398.
27. **Hu, L. T., and H. L. Mobley.** 1990. Purification and N-terminal analysis of urease from *Helicobacter pylori*. Infect. Immun. **58:**992–998.
28. **Jimenez-Pearson, M. A., I. Delaney, V. Scarlato, and D. Beier.** 2005. Phosphate flow in the chemotactic response system of *Helicobacter pylori*. Microbiology **151:**3299–3311.
29. **Kim, E. J., H. J. Chung, B. Suh, Y. C. Hah, and J. H. Roe.** 1998. Transcriptional and post-transcriptional regulation by nickel of *sodN* gene encoding nickel-containing superoxide dismutase from *Streptomyces coelicolor* Muller. Mol. Microbiol. **27:**187–195.
30. **Kim, H., and R. J. Maier.** 1990. Transcriptional regulation of hydrogenase synthesis by nickel in *Bradyrhizobium japonicum*. J. Biol. Chem. **265:**18729–18732.
31. **Labigne A., V. Cussac, and P. Courcoux.** 1991. Shuttle cloning and nucleotide sequences of *Helicobacter pylori* genes responsible for urease activity. J. Bacteriol. **173:**1920–1931.
32. **Livak, K. J., and T. D. Schmittgen.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) method. Methods **25:**402–408.
33. **Maier, R. J.** 2005. Use of molecular hydrogen as an energy substrate by human pathogenic bacteria. Biochem. Soc. Trans. **33:**83–85.
34. **Marshall, B. J., D. B. McGechie, P. A. Rogers, and R. J. Glancy.** 1985. Pyloric *Campylobacter* infection and gastroduodenal disease. Med. J. Aust. **142:**439–444.
35. **Marshall, B. J., and J. R. Warren.** 1984. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet **i:**1311–1315.
36. **McGee, D. J., M. L. Langford, E. L. Watson, J. E. Carter, Y. T. Chen, and K. M. Ottemann.** 2005. Colonization and inflammation deficiencies in Mongolian gerbils infected by *Helicobacter pylori* chemotaxis mutants. Infect. Immun. **73:**1820–1827.
37. **Mobley, H. L., R. M. Garner, and P. Bauerfeind.** 1995. *Helicobacter pylori* nickel-transport gene *nixA*: synthesis of catalytically active urease in *Escherichia coli* independent of growth conditions. Mol. Microbiol. **16:**97–109.
38. **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.
39. **Moncrief, M. B., and M. E. Maguire.** 1999. Magnesium transport in prokaryotes. J. Biol. Inorg. Chem. **4:**523–527.
40. **Noel, L., J. C. Leblanc, and T. Guerin.** 2003. Determination of several elements in duplicate meals from catering establishments using closed vessel microwave digestion with inductively coupled plasma mass spectrometry detection: estimation of daily dietary intake. Food Addit. Contam. **20:**44–56.
41. **Pawitan, Y., K. R. K. Murthy, S. Michiels, and A. Ploner.** 2005. Bias in the estimation of false discovery rate in microarray studies. Bioinformatics **21:**3865–3872.
42. **Pfeiffer, J., J. Guhl, B. Waidner, M. Kist, and S. Bereswill.** 2002. Magnesium uptake by CorA is essential for viability of the gastric pathogen *Helicobacter pylori*. Infect. Immun. **70:**3930–3934.
43. **Pinheiro, J., and D. Bates.** 2000. Mixed-effect models in S and S-plus. Springer, Berlin, Germany.
44. **Sabarth, N., S. Lamer, U. Zimny-Arndt, P. R. Jungblut, T. F. Meyer, and D. Bumann.** 2002. Identification of surface proteins of *Helicobacter pylori* by selective biotinylation, affinity purification, and two-dimensional gel electrophoresis. J. Biol. Chem. **277:**27896–27902.
45. **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.
46. **Spohn, G., A. Danielli, D. Roncarati, I. Delaney, R. Rappuoli, and V. Scarlato.** 2004. Dual control of *Helicobacter pylori* heat shock gene transcription by HspR and HrcA. J. Bacteriol. **186:**2956–2965.
47. **Sunderman, F. J.** 1993. Biological monitoring of nickel in humans. Scand. J. Work Environ. Health **19**(Suppl.)**:**34–38.
48. **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. Karp, 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.

49. **Tsuda, M., M. Karita, M. G. Morshed, K. Okita, and T. Nakazawa.** 1994. A urease-negative mutant of *Helicobacter pylori* constructed by allelic exchange mutagenesis lacks the ability to colonize the nude mouse stomach. Infect. Immun. **62:**3586–3589.
50. **van Vliet, A. H., F. D. Ernst, and J. G. Kusters.** 2004. NikR-mediated regulation of *Helicobacter pylori* acid adaptation. Trends Microbiol. **12:**489–494.
51. **van Vliet, A. H., 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.
52. **Velayudhan, J., N. J. Hughes, A. A. McColm, J. Bagshaw, C. L. Clayton, S. C. Andrews, and D. J. Kelly.** 2000. Iron acquisition and virulence in *Helicobacter pylori*: a major role for FeoB, a high-affinity ferrous iron transporter. Mol. Microbiol. **37:**274–286.
53. **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.