Iron-Responsive Regulation of the *Helicobacter pylori* Iron-Cofactored Superoxide Dismutase SodB Is Mediated by Fur

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Maintaining iron homeostasis is a necessity for all living organisms, as free iron augments the generation of reactive oxygen species like superoxide anions, at the risk of subsequent lethal cellular damage. The iron-responsive regulator Fur controls iron metabolism in many bacteria, including the important human pathogen *Helicobacter pylori*, and thus is directly or indirectly involved in regulation of oxidative stress defense. Here we demonstrate that Fur is a direct regulator of the *H. pylori* iron-cofactored superoxide dismutase SodB, which is essential for the defense against toxic superoxide radicals. Transcription of the *sodB* gene was iron induced in *H. pylori* wild-type strain 26695, resulting in expression of the SodB protein in iron-replete conditions but an absence of expression in iron-restricted conditions. Mutation of the *fur* gene resulted in constitutive, iron-independent expression of SodB. Recombinant *H. pylori* Fur protein bound with low affinity to the *sodB* promoter region, but addition of the iron substitute Mn^{2+} abolished binding. The operator sequence of the iron-free form of Fur, as identified by DNase I footprinting, was located directly upstream of the *sodB* gene at positions –5 to –47 from the transcription start site. The direct role of Fur in regulation of the *H. pylori* *sodB* gene contrasts with the small-RNA-mediated *sodB* regulation observed in *Escherichia coli*. In conclusion, *H. pylori* Fur is a versatile regulator involved in many pathways essential for gastric colonization, including superoxide stress defense.

The human gastric pathogen *Helicobacter pylori* is the causative agent of gastritis and peptic ulcer disease, and infection with *H. pylori* is associated with the development of adenocarcinoma of the distal stomach (20, 34). About one-half of the world's population is infected with *H. pylori*, making it a very successful pathogen. This bacterium colonizes the mucus layer overlaying the gastric epithelial cells, and in this niche it is exposed to hostile environmental conditions caused by the acidic pH and by an active immune response (34). The extraordinary success of *H. pylori* in its hostile niche is indicative of very effective adaptation to these conditions.

The strong immune response of the host induces oxidative stress in *H. pylori* (34). In addition, reactive oxygen species like superoxides are also generated during bacterial respiration and metabolism (33). In view of the microaerophilic requirements of this organism and its low oxygen tolerance, it is not surprising that enzymes involved in the detoxification of reactive oxygen species are important *H. pylori* colonization factors. In *H. pylori* this is demonstrated by the inability of mutants with

mutations in genes encoding components or regulators of detoxification enzymes to colonize the gastric environment in animal models (1, 3, 8, 10, 16, 17, 26, 27, 30, 41, 42).

Iron and oxidative stress defense are intimately linked, as iron potentiates the formation of toxic oxygen radicals through the Fenton and Haber-Weiss reactions (24). Therefore, the modulation of intracellular iron levels is of critical importance in oxidative stress defense (15). In many bacteria intracellular iron levels are controlled by the ferric uptake regulator Fur, which acts as a transcriptional repressor protein that displays iron-dependent binding to conserved DNA sequences (Fur boxes) located in the promoters of iron-regulated genes (15). In *H. pylori*, Fur displays differential binding to promoters depending on the presence or absence of the iron cofactor. As in other bacteria (15), the iron-complexed form of Fur in *H. pylori* binds to promoters of iron uptake genes, thus repressing iron uptake in iron-replete conditions (11, 39). Uniquely, the iron-free form of *H. pylori* Fur (apo-Fur) is also able to bind promoters, as was exclusively shown for the *pfr* gene, which encodes the *H. pylori* iron storage protein Pfr (4, 12, 40). Effectively, the binding of apo-Fur to the *pfr* promoter results in repression of ferritin expression in iron-restricted conditions (4, 40). In addition to regulation of iron metabolism, *H. pylori* Fur has been implicated in regulation of acid resistance (6) and oxidative stress resistance (3, 10, 16).

H. pylori expresses only a single superoxide dismutase (SOD), the iron-cofactored SodB protein (29, 32). Expression of SodB is essential for gastric colonization by *H. pylori* and is

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TABLE 1. Oligonucleotide primers used in this study

Primer	Sequence (5'→3') ^a
SodB-F1	GCTAAAGACAGCATGGGAGA
SodB-R-T7	<u>CTAATACGACTCACTATAGGGAGATCCA</u> CTGATCCTAAGCCTTC
TagD-R1	CTTTATCGCCACCTTTAAGGCT
SodB-R1	ATGGTGGAAATCAAACGCTACAG

^a Primer sequences were derived from the *H. pylori* 26695 genome sequence (35). Underlining indicates a 5' extension with a T7 promoter sequence for creation of an antisense RNA probe.

also required for growth under microaerophilic conditions (30). Recently, it was shown that expression of the *sodB* gene is subject to regulation in response to varying environmental conditions, including iron (14, 19). In this study we demonstrated that Fur mediates iron-responsive regulation of *sodB* expression in *H. pylori* by direct binding of apo-Fur to the *sodB* promoter region. To our knowledge, this is the first description of regulation of oxidative stress defense by apo-Fur.

MATERIALS AND METHODS

Bacterial strains, plasmids, media, and growth conditions. *H. pylori* strain 26695 (35) and an isogenic *fur* mutant (6, 39) were routinely cultured on Columbia agar plates supplemented with 7% saponin-lysed horse blood and Dent selective supplement (Oxoid) at 37°C under microaerophilic conditions (10% CO₂, 5% O₂, and 85% N₂). Broth cultures were grown in brucella broth (Difco) supplemented with 3% newborn or fetal calf serum (Life Technologies, Breda, The Netherlands) (BBN) and were shaken continuously at 40 rpm. Iron restriction was achieved by supplementing BBN with desferal (deferrioxamine mesylate; Sigma) at a final concentration of 20 µM (39). Iron-replete conditions were achieved by supplementing desferal-treated BBN with ferric chloride (Sigma) at a final concentration of 100 µM (39).

Two-dimensional (2-D) polyacrylamide gel electrophoresis. Cells were harvested by centrifugation. After removal of the supernatant, the pellets were washed with phosphate-buffered saline (pH 7.3), resuspended in 10 µl lysis buffer A, which contained 8 M urea, 4% (wt/vol) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate (CHAPS), and 40 mM Tris, and incubated for 45 min on a Eppendorf shaker. Then 450 µl of reswelling solution (8 M urea, 2 M thiourea, 20 mM dithiothreitol [DTT], 1% [wt/vol] CHAPS, 0.52% Pharmalyte [pH 3 to 10]) was added. After vortexing, cell debris was removed by centrifugation at 18,186 × *g* for 10 min at room temperature. The supernatant was used for rehydration of IPG strips in the pH range from 3 to 10 (Amersham Biosciences, Uppsala, Sweden). Proteins were subsequently size separated using the Investigator 2-D electrophoresis system (Genomic Solutions, Chelmsford, MA) as described previously (9), fixed with 40% (vol/vol) methanol–7% (vol/vol) acetic acid, and stained with SYPRO Ruby protein gel stain (Molecular Probes, Eugene, OR). Fluorescence was detected using a Storm 860 PhosphorImager (Molecular Dynamics, Sunnyvale, CA) at a resolution of 200 µm. The 2-D gel image analysis was performed with the DECODON Delta 2-D software (<http://www.decodon.com>), which is based on dual-channel image analysis (5). Protein spots of interest were cut out from the 2-D gel after staining. Mass spectrometry was carried out using a matrix-assisted laser desorption/ionization—time of flight (MALDI-TOF) mass spectrometer (Voyager DE-STR; PerSeptive Biosystems). Peptide mass fingerprints were analyzed by using the MS-Fit software (<http://prospector.ucsf.edu>).

Purification and analysis of RNA. Total RNA was isolated using Trizol (Gibco) according to the manufacturer's instructions. The amount of RNA was determined spectrophotometrically. RNA electrophoresis and blotting onto nylon membranes were carried out as described previously (37). Immediately after transfer, the membranes were stained with methylene blue to confirm the integrity of the RNA samples and to confirm loading of equal amounts of RNA based on the relative intensities of the 16S and 23S rRNA (37). A digoxigenin (DIG)-labeled *sodB*-specific RNA probe was synthesized by *in vitro* transcription using T7 RNA polymerase (Roche Diagnostics), and a PCR product was amplified using primers SodB-F1 and SodB-R-T7 (Table 1). Hybridization of DIG-labeled probes was visualized using a DIG detection kit (Roche Molecular Biochemicals) and the chemiluminescent substrate CDP-Star.

The transcription start site of the *sodB* gene of *H. pylori* 26695 was determined by primer extension analysis (13). Briefly, approximately 5 to 7 µg of total RNA of *H. pylori* wild-type strain 26695 and the isogenic *fur* mutant was incubated with 50 pmol of 5'-DIG-end-labeled primer SodB-R1 (Table 1) and avian myeloblastosis virus reverse transcriptase (Promega). Primer extension products were separated on an 8% polyacrylamide–8 M urea gel and blotted onto a nylon membrane (Roche), and this was followed by chemiluminescent DIG detection.

Electrophoretic mobility shift assay. Recombinant *H. pylori* Fur protein was produced in *Escherichia coli* and purified as previously described (38, 40). The *sodB* promoter region was PCR amplified with primers TagD-R1 and DIG-labeled SodB-R1 (Table 1), which flank the 374-bp *tagD-sodB* intergenic region. Electrophoretic mobility shift assays were performed with two independent isolations of recombinant Fur protein as described previously (38). Briefly, 22 pM of *sodB* promoter DNA was mixed with recombinant Fur protein at concentrations ranging from 0 to 4,500 nM. Protein and DNA were mixed in binding buffer (24% glycerol, 40 mM Tris-Cl, pH 8.0, 150 mM KCl, 2 mM DTT, 600 µg/ml bovine serum albumin, 50 ng herring sperm DNA) in a 20-µl (final volume) mixture and incubated at 37°C for 30 min. As indicated below, manganese chloride (MnCl₂) (Sigma) or EDTA was added to a final concentration of 200 µM. Samples were subsequently separated on a 5% polyacrylamide gel in running buffer (25 mM Tris, 190 mM glycine) for 30 min at 200 V. The gel was then blotted onto a nylon membrane (Roche Molecular Biochemicals), and this was followed by chemiluminescent detection of DIG-labeled DNA. To calculate the binding affinity of Fur for the promoter region of *sodB*, the autoradiograph was digitalized using a Canon CanoScan 5200F scanner at 300 dots per inch and analyzed by densitometry using the Kodak 1D image analysis software, version 3.5.

DNase I footprinting. DNase I footprinting was performed using 440 pM of the DIG-labeled *tagD-sodB* intergenic region, which was mixed with 0, 2.3, 4.6, 6.9, 9.2, or 16 µM Fur protein in DNase binding buffer (10 mM Tris-HCl, pH 8, 50 mM NaCl, 10 mM KCl, 1 mM DTT, 0.1% NP-40, 10% glycerol, 1 µg herring sperm DNA [12]). Reactions were carried out in the presence of 200 µM EDTA, and the mixtures were incubated 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 (12). Subsequently, the fragments were separated on a 7% polyacrylamide–8 M urea sequencing gel. Gels were blotted onto a nylon membrane (Roche), and this was followed by chemiluminescent DIG detection (39).

RESULTS

Iron-responsive expression of SodB is mediated at the transcriptional level. Using DNA array-based transcriptional profiling, the *sodB* gene was recently identified as a member of a regulon comprising 15 *H. pylori* genes which display Fur-dependent, iron-induced transcription (14), similar to the previously described regulation of the *pfr* gene (4, 12). This regulatory pattern was further characterized for the *sodB* gene using Northern hybridization with RNA purified from *H. pylori* wild-type strain 26695 and an isogenic *fur* mutant, both grown in iron-restricted and iron-replete conditions (Fig. 1A). The *sodB*-specific probe hybridized to a single approximately 0.7-kb monocistronic mRNA (29). In the wild-type strain, this mRNA was detected after growth in iron-replete conditions (with Fe) and not after growth in iron-restricted conditions (without Fe), whereas the *sodB* mRNA was present in the *fur* mutant independent of iron availability (Fig. 1A) at levels even higher than those in the wild-type strain.

The transcription start site of the *sodB* gene in *H. pylori* strain 26695 was identified using primer extension analysis (Fig. 1B). There was only a single primer extension product, located at the A residue 21 bp upstream of the ATG start codon, and this matched the transcription start reported previously for *H. pylori* strain 60190 (29) despite the presence of three sequence differences in the *sodB* promoter sequences of the two strains. In accordance with the Northern hybridization data (Fig. 1A), the primer extension product could be detected

FIG. 1. Expression of the *sodB* gene in *H. pylori* strain 26695 is iron induced and Fur repressed. (A) Northern hybridization with a probe specific for the *sodB* gene and RNA purified from *H. pylori* wild-type strain 26695 (wt) and *fur* mutant (*fur*) cells grown in iron-restricted (–Fe) and iron-replete (+Fe) conditions. The positions of the specific mRNAs are indicated on the right, and the positions of the RNA size markers are indicated on the left. Staining of RNA by methylene blue was included for comparison of RNA amounts. (B) Identification of the *H. pylori* *sodB* transcription start site by primer extension analysis, using RNA purified from *H. pylori* wild-type strain 26695 (wt) and *fur* mutant (*fur*) cells grown in iron-restricted (–Fe) and iron-replete (+Fe) conditions. The sequence of the corresponding region is displayed on the left, with the +1 nucleotide and the –10 promoter sequence indicated. (C) Iron- and Fur-responsive regulation of SodB at the protein level. Lysates of *H. pylori* wild-type strain 26695 (wt) and *fur* mutant (*fur*) cells, grown in iron-restricted (–Fe) and iron-replete (+Fe) conditions, were separated on 2-D protein gels. Only the relevant part of the protein gels (molecular masses [MW], 20 to 30 kDa; pI 6 to 7) is shown, and the position of the iron- and Fur-repressed SodB protein (as identified by MALDI-TOF mass spectrometry) is circled.

in the wild-type strain only after growth in iron-replete conditions, whereas it was constitutively present in the *fur* mutant (Fig. 1B).

Iron- and Fur-responsive regulation of the *sodB* gene was confirmed at the protein level by two-dimensional protein gel electrophoresis (Fig. 1C). The SodB protein migrated according to its predicted molecular mass (~24 kDa) and pI (pI ~6.4) (18, 31), and it was positively identified using MALDI-TOF mass spectrometry. The SodB protein was expressed in the wild-type strain only after growth in iron-replete conditions, and it was absent in iron-restricted conditions. Conversely, in the *fur* mutant strain the SodB protein was expressed independent of iron availability (Fig. 1C).

The Fur repressor mediates direct regulation of the *H. pylori* *sodB* gene. To investigate whether the iron-responsive regulation of *sodB* expression is directly or indirectly mediated by Fur, we performed an electrophoretic mobility shift assay with the *sodB* promoter region, using recombinant *H. pylori* Fur protein. Manganese was used as a substitute for the iron cofactor, as it is more stable under the assay conditions used and has been shown to function like iron under in vitro binding conditions (15). In the absence of manganese, Fur caused a shift of mobility of the *sodB* promoter (Fig. 2A). Addition of manganese to the binding reaction mixture abolished the mobility shift (Fig. 2A), indicating that only the metal-free form of Fur (apo-Fur) is able to interact with the *sodB* promoter, which is consistent with the transcriptional regulatory pattern. The affinity of *H. pylori* apo-Fur for the *sodB* promoter region was low, and the K_d value was ~260 nM at a DNA concentration of 22 pM (Fig. 2B and C).

Fur binds to an operator sequence located at positions –5 to –47 in the *sodB* promoter. The Fur binding sequence in the *sodB* promoter region was localized using DNase I footprinting (Fig. 3A). Addition of apo-Fur led to the protection of two regions in the *sodB* promoter, which were separated by a DNase I hypersensitivity site located at position –24 (Fig. 3A). The two protected regions span the area from nucleotide –5 to nucleotide –47 relative to the transcription start site, overlapping the –10 and –35 region of the *sodB* gene (Fig. 3B). While the regulatory pattern of *sodB* regulation resembles that of the *pfr* gene, the apo-Fur binding site in the *sodB* promoter exhibits only poor sequence homology (Fig. 3C) with the two high-affinity binding sites present in the *pfr* promoter region (12), which may explain the relatively low affinity of apo-Fur for the *sodB* promoter.

DISCUSSION

The versatility of iron in redox reactions has resulted in extensive biological use of iron as a cofactor of enzymes, but iron also potentiates the formation of reactive oxygen species like superoxides. Therefore, cells contain mechanisms for protection against iron-associated oxidative stress, and these mechanisms include detoxifying enzymes like catalases and superoxide dismutases, as well as proteins which carefully control cytoplasmic iron homeostasis by balancing the availability of free iron through control of iron acquisition and storage. As the central regulator of iron homeostasis in many bacteria, the Fur repressor often acts both directly and indirectly as a regulator of oxidative stress defense (2, 15, 33).

FIG. 2. Regulation of *sodB* transcription is mediated by direct binding of the *H. pylori* Fur protein to the *sodB* promoter. (A) Electrophoretic mobility shift assay using recombinant *H. pylori* Fur protein and DIG-labeled *sodB* promoter DNA (P_{sodB}) isolated from *H. pylori* strain 26695. In the presence of the iron substitute manganese (+Mn²⁺) (bottom panel), Fur was unable to complex with the *sodB* promoter region, and a shift was not observed. Only in the absence of manganese (+EDTA) (top panel) was apo-Fur able to bind to the *sodB* promoter and cause a mobility shift (indicated as a Fur-P_{sodB} complex). The concentrations of Fur are indicated above the lanes, and the concentration of DNA was 22 pM. (B) Determination of the affinity of apo-Fur for the *sodB* promoter sequence. DIG-labeled P_{sodB} (22 pM) was mixed with increasing concentrations of Fur, with EDTA in the buffer. (C) Graphic representation of determination of the K_d of purified *H. pylori* Fur for the *sodB* promoter, using the data in panel B. The relative amounts of P_{sodB} and Fur-P_{sodB} were determined by densitometry.

In *H. pylori*, Fur mediates iron-dependent repression of iron uptake systems, leading to expression of iron uptake proteins only when iron is required (11, 39). Conversely, Fur also mediates repression of iron storage systems in iron-restricted conditions, by repression of ferritin expression (4, 12). The switch between repression and induction of iron uptake is coupled to the iron availability in the cytoplasm; when iron is available, a Fur dimer forms a complex with ferrous iron and binds to Fur binding sequences (Fur boxes) in the promoters of iron uptake genes (15). However, the situation is not as clear for the switch in repression and induction of ferritin-mediated iron storage; while iron induction of ferritin expression is found in several bacteria, the role of Fur in this process is not universal. Since *H. pylori* colonizes the gastric mucosa, it is thought to encounter both severe iron restriction by lactoferrin and also periods of iron overload after release of iron from food sources (23). Thus, the ability to regulate genes in response to iron restriction and iron overload is an important feature thought to allow chronic colonization of the gastric niche.

SOD catalyzes the dismutation of O₂⁻ to H₂O₂, which is subsequently removed by catalase. *H. pylori* expresses a single SOD (SodB), which is cofactored by iron (29, 30, 32) and is essential for gastric colonization by *H. pylori* in an animal model (30). An absence of SodB leads to cessation of growth in microaerobic conditions and an increase in the DNA mutation rate, probably caused by oxygen radicals formed by iron via the Haber-Weiss and Fenton reactions (30). In *E. coli*, two cytoplasmic SOD species are present: the manganese-cofactored SodA protein and the iron-cofactored SodB protein. Expression of both the *sodA* and *sodB* genes is regulated by iron; in iron-restricted conditions only the SodA protein is expressed, whereas the SodB protein is expressed in iron-replete conditions (25). Iron-responsive repression of the *sodA* gene is mediated directly by Fur, while iron-responsive induction of the *sodB* gene is indirectly affected by Fur via the RyhB small RNA (22). Transcription of the RyhB small RNA is Fur dependent, and once transcribed, RyhB can bind to complementary sequences in the 5' end of the *sodB* mRNA, blocking translation and making the mRNA unstable (21, 22).

Regulation of the oxidative stress defense in *H. pylori* has not been studied in detail, but recent studies indicated that expression of antioxidant genes is controlled by transcriptional regulation through an intricate regulatory network (3, 10, 16, 19, 40). This is exemplified by a compensatory increase in expression of the antioxidant protein NapA upon mutation of the antioxidant enzyme alkyl hydroperoxide reductase (AhpC) (26). In contrast to *E. coli*, regulation by small RNAs has not been described for *H. pylori*, but it could also not explain the regulation of the *H. pylori* ferritin gene *pfr* (4, 12) or the *sodB* gene (this study). As in *E. coli*, expression of both Pfr and SodB is iron induced, but in contrast to *E. coli*, the mRNA levels of *pfr* and *sodB* are constitutively high in the *fur* mutant (Fig. 1A) (4). This expression pattern of SodB suggested a direct role for apo-Fur in regulation of the *sodB* gene, as previously described for the *pfr* gene (12, 40).

Direct and sequence-specific binding of *H. pylori* apo-Fur to the *H. pylori* *sodB* promoter region was confirmed using an electrophoretic mobility shift assay and DNase I footprinting assays (Fig. 2 and 3). apo-Fur bound to the region overlapping the -10 and -35 promoter sequences present in the *sodB*

FIG. 3. Identification of the operator sequence for apo-Fur in the *H. pylori* 26695 *sodB* promoter. (A) DNase I footprinting assay using 440 pM DIG-labeled *H. pylori* 26695 *sodB* promoter DNA and increasing concentrations apo-Fur. The positions of the -10 and -35 promoter sequences located in the *sodB* promoter are indicated on the left, and the positions of the two protected regions (located at positions -5 to -23 and -25 to -47) and the DNase I hypersensitive site (at position -24) are indicated on the right. The concentrations of Fur protein used in lanes 1 to 6 were 0, 2.3, 4.6, 6.9, 9.2, and 16 μ M, respectively. (B) Graphic representation of the *sodB* promoter with the location and sequence of the apo-Fur binding site indicated. The DNase I hypersensitivity residue is indicated between the two binding sites by a black background. The -10 and -35 promoter sequences are underlined in the binding sequence. RBS, ribosome binding sequence. (C) Alignment of the proposed binding sequence for apo-Fur in the *H. pylori* 26695 *sodB* promoter with the high-affinity binding sequences in the *H. pylori* *pfr* promoter (*pfr* boxes I and II) (12). Residues in the *sodB* sequence identical to residues in both *pfr* binding sequences are indicated by a black background, and residues identical to residues in only one of the *pfr* binding sites (12) are indicated by a grey background.

promoter (Fig. 3A and B). The affinity of *H. pylori* apo-Fur for the *sodB* promoter was surprisingly low ($K_d = 260$ nM) compared to the affinity of metal-cofactored *H. pylori* Fur for the *amiE* promoter (~ 10 nM, calculated from the data of van Vliet et al. [38]). This low affinity may have biological significance. Genes involved in iron metabolism need to be tightly regulated to prevent iron surplus in the cell and therefore creation of Haber-Weiss and Fenton reactions. In contrast, the SodB protein is the only defense against superoxide stress in *H. pylori* (30), and thus its expression should not be repressed unless *H. pylori* encounters such severe iron restriction that even activat-

ing SodB enzyme is not feasible. Low-affinity binding of Fur to the *sodB* promoter is one way of achieving such regulation.

The DNase I footprinting pattern obtained for the *sodB* promoter resembled those identified for the *pfr* promoter, as there was a DNase I hypersensitivity site between the two protected regions (Fig. 3). However, the operator sequence present in the *sodB* gene displayed only very limited sequence homology to those identified in the *pfr* promoter. The limited availability of binding sequences of apo-Fur currently precludes definition of a consensus sequence. This could be resolved by studying additional promoters which are regulated by apo-Fur, and several candidate promoters have been described recently (14), including the *hydABC* operon encoding the iron- and nickel-cofactored hydrogenase enzyme (28).

Despite its small genome, *H. pylori* is a highly successful colonizer of the human gastric mucosa, persisting lifelong unless it is eradicated by antibiotic treatment (7). The Fur protein, which is well known for its central role in iron homeostasis in bacteria, affects the expression of different pathways involved in normal metabolism, stress resistance, motility, and virulence (4, 8, 12, 36, 38, 39). In our study, we expanded the role of Fur in one of these aspects, the regulation of oxidative (superoxide) stress defense. Fur directly represses the expression of SodB when the iron cofactor is not available, thus not wasting valuable cellular resources. When iron is available, repression is terminated, allowing expression of iron-cofactored SodB in conditions in which the risk of formation of reactive oxygen species is high. This direct role of Fur contrasts with the indirect Fur-mediated regulation of iron-cofactored SOD in *E. coli* (22) and highlights the special aspects of *H. pylori* Fur compared to other eubacterial Fur proteins. In conclusion, this is the first description of a *sodB* gene that is directly regulated by apo-Fur, and thus the mechanism described here is a novel mechanism for regulation of expression of Fe-containing superoxide dismutases in prokaryotes.

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