A Single Nucleotide Change Affects Fur-Dependent Regulation of sodB in H. pylori

Beth M. Carpenter¹, Hanan Gancz¹, Reyda P. Gonzalez-Nieves¹, Abby L. West², Jeannette M. Whitmire¹, Sarah L. J. Michel², D. Scott Merrell¹*

¹Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, Maryland, United States of America, ²Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, Maryland, United States of America

Abstract

*Helicobacter pylori* is a significant human pathogen that has adapted to survive the many stresses found within the gastric environment. Superoxide Dismutase (SodB) is an important factor that helps *H. pylori* combat oxidative stress. *sodB* was previously shown to be repressed by the Ferric Uptake Regulator (Fur) in the absence of iron (apo-Fur regulation) [1]. Herein, we show that apo regulation is not fully conserved among all strains of *H. pylori*. apo-Fur dependent changes in *sodB* expression are not observed under iron deplete conditions in *H. pylori* strains 27, HPAG1, or J99. However, Fur regulation of *pfr* and *amiE* occurs as expected. Comparative analysis of the Fur coding sequence between 27 and 26695 revealed a single amino acid difference, which was not responsible for the altered sodB regulation. Comparison of the *sodB* promoters from 27 and 26695 also revealed a single nucleotide difference within the predicted Fur binding site. Alteration of this nucleotide in 27 to that of 26695 restored apo-Fur dependent sodB regulation, indicating that a single base difference is at least partially responsible for the difference in sodB regulation observed among these *H. pylori* strains. Fur binding studies revealed that alteration of this single nucleotide in 27 increased the affinity of Fur for the *sodB* promoter. Additionally, the single base change in 27 enabled the *sodB* promoter to bind to apo-Fur with affinities similar to the 26695 sodB promoter. Taken together these data indicate that this nucleotide residue is important for direct apo-Fur binding to the *sodB* promoter.

Citation: Carpenter BM, Gancz H, Gonzalez-Nieves RP, West AL, Whitmire JM, et al. (2009) A Single Nucleotide Change Affects Fur-Dependent Regulation of sodB in *H. pylori*. PLoS ONE 4(4): e5369. doi:10.1371/journal.pone.0005369

Editor: Niyaz Ahmed, University of Hyderabad, India

Received August 25, 2008; Accepted March 27, 2009; Published April 28, 2009

This is an open-access article distributed under the terms of the Creative Commons Public Domain declaration which stipulates that, once placed in the public domain, this work may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose.

Funding: Research in the laboratory of D. Scott Merrell is made possible by grants R073LA from USUHS and AI065529 from the NIAID. Contents of this manuscript are the sole responsibility of the authors and do not necessarily represent the official views of the NIH or the DOD. The funders have no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: dmerrell@usuhs.mil

Introduction

*Helicobacter pylori* is an important human pathogen that infects over 50% of the world’s population [2]. While infection is predominantly asymptomatic, this bacterium is associated with development of gastritis, peptic ulcer disease, mucosa-associated lymphoid tissue lymphoma, and gastric adenocarcinoma. Infection often occurs early in childhood and persists throughout a person’s lifetime unless they are treated with specific antibiotics [3]. Given its propensity for chronic colonization and the substantial number of infected individuals, *H. pylori* imposes a significant disease burden worldwide.

This microaerophilic, Gram negative bacterium is interesting in that it colonizes and survives within the gastric mucosa of the human stomach. *H. pylori* is well suited to life within this niche and has many factors that enable it to thrive there [2,4]. One such factor, the Ferric uptake regulator (Fur), functions as a transcriptional regulator that is involved in maintaining iron homeostasis [5]. Iron is essential for bacterial survival and is a co-factor in a variety of proteins; however, iron is redox active and can promote oxidative damage making it imperative that intracellular iron levels are tightly controlled. One particularly deleterious reaction that free iron can promote is reaction with reactive oxygen species (ROS) to form highly reactive hydroxyl radicals via Fenton chemistry. Hydroxyl radicals cause DNA and cellular damage that eventually lead to cell death. Thus, cells must strive to maintain a balance between insufficient and excess iron. Fur is involved in preserving this fine balance in *H. pylori*, and consequently, it is not surprising that fur has been shown to be critical for colonization in both gerbil and murine models of infection [6,7].

Fur is conserved in a wide variety of bacterial species and functions similarly in all of them by repressing gene expression under conditions of sufficient cellular iron. When Fur is bound to its iron (Fe²⁺) co-factor, it binds to specific regions in iron-regulated promoters called Fur Boxes and blocks the binding of RNA polymerase. Genes regulated in this manner are often associated with iron acquisition and are repressed under iron replete conditions to prevent the harmful effects of iron overload. While *H. pylori* Fur has been found to repress a set of genes in its iron-bound state, it has also uniquely been found to repress an additional set of genes in the absence of the iron cofactor, i.e. when Fur is in its apo form. apo-Fur regulation involves repression of an iron storage gene and occurs under iron limited conditions [8]. apo-Fur regulation has not been described for other bacterial species, and given that Fur plays a role in global gene regulation in response to environmental stressors and enhances the fitness of *H. pylori* as a pathogen, functional studies of Fur in *H. pylori* are of particular interest. One gene known to be repressed by apo-Fur in *H. pylori* is *sodB*.
SodB is linked to iron metabolism, but is involved with the oxidative stress response, is superoxide dismutase (sodB) [1]. SodB was first identified in H. pylori in 1993 and was shown to be iron co-factor of the Escherichia coli FeSod with 53.5% identity between the two proteins [9]. However, unlike E. coli FeSod, which is localized within the cytosol of the bacterium, H. pylori SodB is associated with the cell surface [9]. SodB is the only identified Sod in H. pylori and has been shown to be critical for survival in vivo [10]. Interestingly, H. pylori sodB deficient mutants have been shown to harbor more free iron within their cells than WT bacteria [11].

Globally, Sods are responsible for combating oxidative stress (both internal and external) by converting superoxide radicals into hydrogen peroxide and oxygen. Superoxide radicals are formed as a by-product of metabolism and, if left unchecked, can react with ferric iron (Fe3+) to form hydrogen peroxide, which in turn feeds the Fenton Reaction [12] and is detrimental to the cell. Sods prevent the interaction of iron and superoxide radicals as well as block the formation of hydroxyl radicals from hydrogen peroxide [12]. In this way, the role of Fur as the primary regulator of iron uptake and the role of SodB as the primary defense against superoxide radicals in H. pylori are linked. In keeping with this, sodB has been shown to be regulated by apo-Fur such that it is repressed under circumstances where iron is severely limited [1]. This regulation appears to be direct since Electrophoretic Mobility Shift Assays showed that Fur specifically binds to the sodB promoter in the absence of iron [1]. Herein we describe a series of experiments that define a single polymorphic nucleotide within the H. pylori sodB promoter that is important for apo-Fur dependent regulation. Moreover, we show that alterations in this single base result in strain specific responses to iron limitation.

Materials and Methods

Bacterial strains and growth

Strains and plasmids used in this study are listed in Table 1, and primer sequences are listed in Table 2. Strains of H. pylori were maintained as frozen stocks at −80°C in brain heart infusion broth (BD) supplemented with 10% fetal bovine serum (Gibco) and 20% glycerol (EMD Chemicals, Inc.). Bacterial strains were grown on horse blood agar (HBA) plates which contained 4% Columbia agar base (Neogen Corporation), 5% defibrinated horse blood (HemoStat Laboratories, Dixon, CA), 0.2% β-cyclodextrin (Sigma), 10 μg/ml vancomycin (Amresco), 5 μg/ml cefalosporin (Sigma), 2.5 U/ml polymyxin B (Sigma), 5 μg/ml trimethoprim (Sigma), and 8 μg/ml amphotericin B (Amresco). Liquid cultures of H. pylori were grown in brucella broth (Neogen Corporation) supplemented with 10% fetal bovine serum and 10 μg/ml vancomycin at 37°C with shaking at 100 rpm. As noted in Table 1, where appropriate, cultures and plates were supplemented with 8 μg/ml chloramphenicol (Cm) (EMD Chemicals, Inc.) and/or 25 μg/ml kanamycin (Kan) (Gibco). In addition, where detailed in the Materials and Methods, some HBA plates contained 5% sucrose (Suc) (Sigma). Both liquid and plate cultures were grown under microaerophilic conditions (5% O2, 10% CO2, and 85% N2) generated with an Anoxomat gas evacuation and replacement system (Spiral Biotech) in gas evacuation jars.

H. pylori strains used in this study are all derivatives of G27 [13] and 26695 [14,15], with the exception of WT H. pylori J99 [16] and HPAG1 [17]. A fur (HP1027) mutant of G27, DSM300, was utilized in this work and contains a deletion insertion of the fur coding sequence with the cat gene from Campylobacter coli conferring Cm resistance as previously described [18]. This AHP1027:cat construct was also naturally transformed into 26695 to create an analogous fur mutation in this strain background and is called DSM357. Exponential phase cultures were grown for 20 hrs, and stationary phase cultures were grown for 44 hrs.

Creation of the sodB promoter fusion plasmid

A transcriptional fusion of the sodB (HP0389) promoter to the promoterless gfpmut3 on the transcriptional reporter plasmid, pTM117, was constructed as previously described [18]. Briefly, the sodB promoter of WT G27 was PCR amplified using sodB-F1 and sodB-R1 primers, which incorporate SacII and BamHI restriction sites, respectively. The resulting PCR fragment was subcloned into pGEM-T Easy (Promega) and digested with SacII (New England

<Table 1. Plasmids and strains used in this study.</Table>

| Plasmid or strain | Description | Reference |
|-------------------|-------------|-----------|
| Plasmids          |             |           |
| pTM117            | Modified pH666 to include E. coli origin and rap gene, aphA-3 cassette (Kan'), multiple cloning site, and a promoterless gfpmut3 gene | [18] |
| pDSM236           | pTM117 sodB promoter::gfpmut3fusion | This study |
| pDSM368           | pTM117 pfr promoter::gfpmut3fusion | [18] |
| pKF5-II           | pEF::kan-sacB | [19,20] |
| pDSM386           | pGEM-T Easy::fur | This study |
| pDSM387           | pGEM-T Easy::fur::kan-sacB | This study |
| pDSM469           | pGEM-T Easy::sodB | This study |
| pDSM475           | pGEM-T Easy::sodB::kan-sacB | This study |
| pDSM481           | pGEM-T Easy::sodB::kan-sacC::A | This study |
| pDSM429           | pGEM-T Easy::26695 fur | This study |
| pDSM430           | pET21A::26695 fur | This study |
| pKD4              | kan template plasmid | [22] |
| pKD46             | Red recombinase expression plasmid | [22] |
| H. pylori strains |             |           |
| G27               | WT. H. pylori | [13] |
| DSM300            | fur::cat/Cm' | [18] |
| 26695             | WT. H. pylori | [14,15] |
| DSM357            | 26695::fur::cat/Cm' | This study |
| DSM238            | G27(pDSM236), Kan' | This study |
| DSM308            | DSM300(pDSM236), Kan' Cm' | This study |
| DSM369            | DSM300(pDSM368), Kan' | [18] |
| DSM370            | DSM300(pDSM368), Kan' | [18] |
| DSM391            | fur::kan-sacB, Kan' | Suc' | This study |
| DSM403            | fur, fur 26695, Suc' Kan' | This study |
| DSM480            | fur::kan-sacB, Kan' | Suc' | This study |
| DSM491            | fur::sodB::kan-sacB, Kan' | Suc' | This study |
| J99               | WT. H. pylori | [16] |
| HPAG1             | WT. H. pylori | [17] |
| E. coli strains   |             |           |
| DSM328            | K12(pKD46), Amp', Temp' | [22] |
| DSM355            | K12 Δfur, Kan' | This study |
| DSM326            | BL21 DE3 Rosetta/pLysS, Cm' | This study |
| DSM365            | BL21 DE3 Rosetta/pLysS Δfur, Kan', Cm' | This study |
| DSM431            | BL21Δfur (pDSM430) Amp', Cm', Kan' | This study |

doi:10.1371/journal.pone.0005369.t001

PloS ONE | www.plosone.org 2 April 2009 | Volume 4 | Issue 4 | e5369
Biolabs) and BamHI (Invitrogen). The resulting promoter fragment was then ligated into the appropriately digested pTM117 vector to create pDSM236. The fusion was confirmed by PCR amplification with sodB-F1 and gfp-1 primers and by sequencing with the aphA3-2 primer. pDSM236 was naturally transformed into WT G27 and DSM300, and transformants were selected on HBA plates containing 25 μg/ml Kan and 25 μg/ml Kan plus 8 μg/ml Cm, respectively. The WT strain bearing pDSM236 was designated DSM238, and DSM300 bearing pDSM236 was designated DSM308.

**GFP reporter assays**

The ability of the sodB transcriptional fusion to drive the expression of GFP was assessed using flow cytometry as described.

---

**Table 2. Primers used in this study.**

| Primerb | Sequence (5’-3’)a | Reference |
|---------|------------------|-----------|
| sodB promoter primers | | |
| sodB-F1 (SacII) | CCAGCCGCGCATTTGACCAATTTTCA | This study |
| sodB-R1 (BamHI) | GATCCGCAACTCTCGTTAATGAAAC | This study |
| Screening and Sequencing primers | | |
| gfp-1 | AAGTGCCTGGTCTCATGTT | [18] |
| apha3-2 | CCGTATTTTCAATTTGACC | [18] |
| sacBSCN-F2 | CGAATCAATGCTAAGAC | This study |
| HpKanSacSCN-R | GGGAACTCTCGTTAATGG | This study |
| HpsodBSCN-R | GCTCGCTCTTAAACTCAACC | This study |
| Cloning primers | | |
| FurCF (XbaI) | CTCTAGAAGGCTCAGCTACCTACCCTATT | [18] |
| HpUKanSacCF (Xhol, Smal) | CTCTTGCTACTTCTTACAACCAACACCCGGGAGGCTGAGCTGGATATCTCTTCATTACGG | This study |
| HpsodBSCF (Xhol, Smal) | CGGATAAGAAAGATATGACCGCATGCCGCTCCCGGGTGTTGTTGATAGAATTCGCAAGAG | This study |
| HpKanSacR | CGCAGCGATAAAAGGGGCTTGGT | This study |
| FurCR (SalI) | GTCGAGAAGACTTCTACCCTGAAAGCC | [18] |
| USoD-F | GCTTTATGCGGCCACTTCCAG | This study |
| USoD-R | CCAATAGGTGAGCTAGCTGTGTTTATCTCTTTGTGATAAGG | This study |
| DSoD-F | CTAATCAACAGGGAGAGAAATACTTGCTGAGCTCCCGGGTGTTGTTGATAGAATTCGCAAGAG | This study |
| DSoD-R | GCCAGGAAATTTGAATGGTATCC | This study |
| SedBmt-R | GATACCTCTTATGTAATC | This study |
| SedBmt-F | GATACCAATAAGGGCTATC | This study |
| HP_Fur_expression F2 (Ndel) | CATATGAAAGGAAGTGAAGCATTTTGAATCCATT | This study |
| HP_Fur_expression R2 (XhoI) | CTCTAGGTTTACCTTCAGCTCCCTCGTTG | This study |
| Red_EC_Fur_F | GAGTCTGAATCTCGTGTTTCTTTCATTTTCCTTTCGCTGGTGCTGCTTC | This study |
| Red_EC_Fur_R | TCATATGGGCGGATTTATATGAAAAATTGATGCAATACCATGATATCCTTCTCATTGTC | This study |
| RPA primers | | |
| amiE-RPA-F | GCTTTTCGCGCGTGGAT | [7] |
| amiE-RPA-R | GATTTCGCGCGTGGAT | [7] |
| pfr-RPA-F | GCGGTGAGAGATGACGG | [18] |
| pfr-RPA-R | GTTATGAGACAAAAAACAA | [18] |
| sodB-RPA-F | AAGCGCTGAGAGTGTTGATT | This study |
| sodB-RPA-R | CCCATACGCAACAGCCA | This study |
| fur RPA F | GAGTTTCTAGGAGATCTCATC | [18] |
| fur RPA R | GTGATCATGGGTCTTTTACG | [18] |
| EMSA primers | | |
| G27 sodB EMSA-F | CTACAAAATTTGCAACG | This study |
| 26695 sodB EMSA-F | CCAACAAAATTGCAACG | This study |
| sodB EMSA-R | GCAACTCTGTAATGTAACG | This study |
| rpoB EMSA-F | CCAAGAGGGAAGAGCG | This study |
| rpoB EMSA-R | CCTCTCAGAATCTCTTCAACG | This study |

*a Restriction endonuclease sites are underlined, and linker bases are in bold type.  
*b Important restriction sites are included in parentheses.  
doi:10.1371/journal.pone.0005369.t002
Creation of a “−5 bp swap” mutation in the sodB promoter

The sodB promoter from G27 was sequenced using primers USod-F and DSod-R and compared to the known sequence of the sodB promoter from 26695 [14]. This comparison revealed a single base pair (bp) difference within the predicted Fur Box [1] at the −5 position relative to the start of transcription. The “−5 bp swap” mutation within the sodB promoter of G27 was created using SOE PCR and the kan-sacB cassette from pKSF-II. A 297 bp region upstream and a 329 bp region downstream of sodB were PCR amplified from G27 using primer pairs USod-F and USod-R and DSod-F and DSod-R, respectively. USod-R and DSod-F contain Xhol and Smal restriction endonuclease sites to allow for the directional cloning of the kan-sacB fragment. The upstream and downstream products were purified and mixed in a SOE PCR reaction with the USod-F and DSod-R primers. The resulting 626 bp SOE PCR product was subcloned into pGEM-T Easy to create pDSM469. pDSM470 and pKSF-II were each sequentially digested with XhoI and Smal, and the resulting fragments were ligated to create pDSM475. The sodB promoter was naturally transformed into WT G27, and transformants were selected on Kan and then patched to verify sucrose sensitivity. Double crossover homologous recombination of pDSM475 into the G27 chromosome results in the deletion of the sodB gene and replacement with the kan-sacB cassette. The resulting Kan resistant, sucrose sensitive strain, DSM490, was confirmed by PCR with sacBSCN-F2 and HpsodBSCN-R primers, the latter of which lies downstream of sodB.

The −5 bp in the G27 sodB promoter was mutated from a C to an A using SOE PCR. First, primers USod-F and SodBMt-R were used to PCR amplify upstream of the sodB promoter through to the −5 bp and incorporate the C-5A mutation. Second, primers DSod-R and SodBMt-R were used to PCR amplify from the −5 bp through to downstream of the sodB gene and to incorporate the C-5A mutation. These products were purified and combined in a SOE PCR reaction using the USod-F and DSod-R primers. The resulting SOE PCR product was subcloned into pGEM-T Easy. The subcloned sodB−5 bp promoter mutation construct was designated pDSM481 and was confirmed by sequencing with the USod-F and DSod-R primers. pDSM481 was naturally transformed into DSM480 to integrate the sodB−5 bp promoter mutation into the chromosome in place of the kan-sacB cassette. Transformants were selected as detailed above for the creation of DSM403. The resulting Suc resistant, Kan sensitive strain was named DSM491. Proper recombination was confirmed by PCR with the USod-F and DSod-R primers (yielding a 1,262 bp fragment) and by sequencing with both of those primers. DSM491 expresses sodB with the C-5A mutation from its native locus within the G27 chromosome.

RNase protection assays (RPAs)

RPAs were utilized to characterize apo-Fur regulation of sodB in various strains of H. pylori. Two normal (iron replete) media cultures were started for each strain, one for exponential and one for stationary growth phase. Following overnight growth, one half of each exponential phase culture was removed for RNA isolation. The remaining half of each culture was grown in iron limited media (60 μM dpp) and on the following morning one half of the culture was removed for stationary growth phase. Following overnight growth, one half of each strain was grown in iron limited media (60 μM dpp) and on the following morning one half of each culture was removed for RNA isolation. The remaining half of each culture was grown in iron deficient media (60 μM dpp). After overnight growth, one-half of each culture was removed for RNA isolation in exponential phase. The remaining half of the
iron-limited growth culture was allowed to grow overnight and was harvested the following morning for the stationary phase, iron-limited growth RNA samples. RNA was extracted as described previously [21]. RNase Protection Assays (RPAs) were performed as previously described [10] with 1.5 μg of RNA using sodB, pfp, amiE, and/or fur riboprobes that were generated using the primer pairs listed in Table 2. In brief, riboprobes were generated with 50μCi \(^{32}\)PUTP (Perkin-Elmer) and a MaxiScript kit (Applied Biosystems). The RPA III kit (Applied Biosystems) was used for the RPA reactions that were resolved on 5% acrylamide-1\(\times\) Tris-borate-EDTA-8M urea denaturing gels. The gels were exposed to phosphor screens, and the phosphor screens were scanned using a FLA-5100 multifunctional scanner (Fujifilm). Analyses and quantitation of the RPAs were performed using the Multi-Gauge software (version 3.0, Fujifilm). In all cases, three to four biological repeats of each experiment were performed.

**H. pylori Fur Expression and Purification**

*H. pylori* 26695 Fur coding sequence was amplified using primers HP_Fur_expression F2 (NdeI) and HP_Fur_expression R2 (XhoI), and the PCR product was cloned into the pGEM-T easy vector (Promega) to create plasmid pDSM429. pDSM430 was created by proper digestion of pET21A (Novagen) and pDSM429 with NdeI and XhoI and ligation of the gel purified fragments. The Fur coding region in pDSM430 was sequenced to verify the construct. To prevent cross contamination of *H. pylori* recombinant Fur with *E. coli* endogenous Fur, an *E. coli* Bl21 Rosetta *Apo* strain was constructed using the Wanner method [22]. Briefly, the Kan resistance cassette was amplified from pKD4 [22] with primers Red_EC_Fur_F and Red_EC_Fur_R. This PCR product was introduced into arabinose induced *E. coli* K-12 carrying the pKD46 plasmid [22] to create DMS355. DMS365 was created by transduction of DMS326 with P1L4 grown on DMS355. Endogenous *E. coli* Fur deletion was verified by PCR. pDSM430 was introduced into DMS365 to create DMS431, which was used for fur induction. DMS431 was grown to mid log in Luria-Bertani (EMD Chemicals) medium and then induced with 0.5 mM IPTG (isopropyl-D-thiogalactopyranoside) (Sigma) at 30°C for 3 h. The cells were disrupted using French press (Amicon) and crude extracts were prepared from the IPTG-induced cells by centrifugation (5,000 rpm for 30 minutes). Protein purification was performed by fast-protein liquid chromatography; the cytoplasmic protein was first passed through a HiTrap SP column for ion-exchange-based purification with a salt gradient of 25 mM to 500 mM NaCl (obtained by using buffer A [50 mM sodium phosphate, 25 mM NaCl, pH 8.0] and buffer B [25 mM sodium phosphate, 500 mM NaCl, pH 8.0]). Peak fractions containing Fur protein from the ion-exchange procedure were collected and further purified based on size exclusion by using a Sephacryl-200 column (buffer C [50 mM sodium phosphate, 200 mM NaCl, pH 8.0]). Fur was partially concentrated using an Amicon Ultra Centrifugal Filter Device (Millipore) to remove a portion of buffer C. Then an equal volume of EMSA binding buffer (BB) was added to the partially concentrated rFur with an additional 50% glycerol. rFur was further concentrated before being quantitated and stored at −20°C. The final concentration of the rFur stock was 2 mg/mL.

**Electrophoretic Mobility Shift Assays (EMSAs)**

A 120 bp region of the *sodB* promoter (comprising the Fur-box) [1] was PCR amplified using the following template and primer pairs: WT G27 and DMS491 (“−5 bp swap”) with G27 sodB EMSA-F and sodB EMSA-R and WT 26695 with 26695 sodB EMSA-F and sodB EMSA-R. To serve as a negative control in the EMSA studies, a 142 bp region of the *rpoB* promoter was amplified from WT G27 using the rpoB EMSA-F and rpoB EMSA-R primer pairs. Each PCR product was acrylamide gel purified and resuspended in 1× Tris-EDTA (TE) buffer. 150 ng of each promoter region was end labeled with \(^{32}\)P ATP (Perkin Elmer) using T4 polynucleotide kinase (New England Biolabs) as previously described [7]. The unincorporated nucleotide was removed using the MinElute Reaction Clean-up kit (Qiagen), and labeled promoter fragments were eluted twice with 10 μL EB, and 50 μL of apo-BB was added to the eluted product.

EMSA were performed under apo (iron-free) conditions as previously described for WT 26695 *sodB* [1]. Briefly, 1 ng of labeled sodB or rpoB promoter was mixed with 5 μL of the following dilutions of the Fur stock: 1:1,875, 1:3,125, 1:1,562,5, and 1:787,25, and each with 10 μL of 2× apo-BB (24% glycerol, 40 mM Tris, pH 8.0, 150 mM KCl, 2 mM DTT, 600 μg/mL bovine serum albumin, 200 μM EDTA, and 0.1 mg/mL sheared salmon sperm DNA). In addition, a no protein control reaction and a 100 ng cold (unlabeled) DNA competition reaction were performed. The cold competition reaction was performed with the highest concentration of Fur (1:1,875). All reactions were allowed to incubate at 37°C for 30 min. After the incubation, the reactions were separated on a 5% polyacrylamide gel (5% 19:1 acrylamide, 1× Tris Glycine EDTA (TGE) buffer, 2.5% glycerol) for 3 hours at 70 V in 1×TGE buffer. The gels were then exposed to phosphor screens and scanned on a Storm 860 scanner (GE Healthcare). Analysis was performed using ImageQuant version 5.2 software (Molecular Dynamics).

**Competition EMSA Studies**

Competition studies were performed in a manner analogous to the EMSAs. Each labeled sodB promoter fragment was combined with the 1:1,875 dilution of rFur, apo-BB, and either 5 ng, 10 ng, or 25 ng of cold (unlabeled) sodB promoter from each of the three respective strains. A no competitor control was included for each labeled sodB promoter fragment. In this manner, each labeled sodB fragment (WT G27, “−5 bp swap,” and WT 26695) competed for binding to Fur with its own unlabeled sodB fragment as well as to that of the other two strains. The incubations, electrophoresis, and analysis were performed as described for the EMSAs. Binding competition occurs as follows: \(PD_{p2} + D_{p2} + D_{p2} + D_{p2,3}\), where \(P = Fur, D_{p2} = labeled DNA, and D = cold competitor). Thus, if the competitor promoter fragment (D) can bind to Fur (P) with a higher affinity than the labeled promoter (\(D_{p2,3}\)), then an increase in the amount of unbound, labeled promoter (\(D_{p2,3}\)) would be seen. The percent of unbound, labeled sodB promoter was quantitated for each competition EMSA using densitometry as a means of comparing the relative affinity of each promoter fragment for Fur.

**Statistical Analysis**

Two-tailed Student’s t-tests were performed using Microsoft Office Excel 2003.

**Nucleotide sequence accession number**

The nucleotide sequence of the *sodB* promoter is available from GenBank under accession number EU888136. The G27 fur sequence was previously reported [18] and is available as GenBank accession number EF537051.

**Results**

**apo-Fur Regulation in *H. pylori***

In order to study *apo*-Fur dependent regulation in *H. pylori*, the *sodB* and *pfp* promoters from strain G27 were fused to the
promoterless gfpmut3 gene in pTM117. Currently, these promoters represent the only known targets of apo-Fur [1,8]. Given this apo-regulation and since promoter activity can be measured by changes in fluorescence with our system, we expected to see a decrease in GFP fluorescence under iron limited conditions for both promoter fusions. However, as shown in Fig. 1A, the addition of iron chelator resulted in no change in the level of sodB expression. This is in contrast to pfr, where iron depletion resulted in strong repression of pfr expression (Fig. 1B). Both sodB and pfr were upregulated in a fur mutant (Fig. 1A and 1B) suggesting that both genes are repressed by Fur. However, the lack of responsiveness to iron chelation suggested that sodB apo-regulation is not as expected in G27.

Since apo-Fur has been shown to have a lower affinity for the sodB promoter than the pfr promoter, and since the gfpmut3 allele encodes a long-lived GFP variant [23], we reasoned that we might not be able to detect small changes in GFP expression under the control of the sodB promoter under iron limited conditions. Therefore, we performed RPAs to further investigate the discrepancy between our results and results previously reported for sodB regulation in strain 26695 [1]. Additionally, we considered the fact that strain specific differences might be responsible for the discrepancy. Therefore, RPAs using a sodB riboprobe were performed on RNA isolated from WT and Δfur derivatives from both G27 and 26695. pfr and amiE (aliphatic amidase) riboprobes were also used as control apo-Fur and iron-bound Fur regulated target genes, respectively. Fig. 2A shows results for all three riboprobes using RNA isolated from exponential phase cultures. Again, we observed that for G27 the level of sodB expression did not change under iron-limited growth conditions (G) or under a harsher iron-depletion shock condition (S) that was added to ensure robust chelation as compared to normal (N) iron replete conditions.

Examination of sodB expression in 26695 revealed a smaller protected fragment than originally expected. However, sequence analysis revealed that the smaller fragment is due to a small region of mismatch between the sodB mRNA sequence in 26695 and the G27 template DNA used to generate the riboprobe. This mismatch causes a bubble of single stranded RNA to form and thus is subjected to RNase cleavage in the region of mismatch (data not shown). For WT 26695, a 2-fold decrease in sodB expression was achieved under both iron-limited growth and iron-depletion shock conditions, which agrees with the previous report [1]. This change is Fur-dependent as there is no change in sodB expression under either iron depletion condition in the absence of fur.

Since it has been shown that growth phase strongly affects gene expression in H. pylori [21], we performed similar experiments on RNA harvested from stationary phase cultures. As shown in Fig. 2B, we obtained identical results with the exception that the fold decrease seen in sodB expression was less pronounced in 26695 in this growth phase. Again, there was no decrease in sodB expression in G27, indicating that growth phase is not responsible for the differences in our results. Moreover, the difference in sodB regulation between the two strains is not the result of a generalized difference in apo-Fur regulation between G27 and 26695 since the appropriate decrease in pfr expression [8] was observed in both strains under iron-limited growth and iron-depletion shock conditions.

Figure 1. Flow Cytometry analysis of sodB and pfr GFP reporters. Strains bearing sodB::gfpmut3 or pfr::gfpmut3 promoter fusions were grown overnight in either iron replete or iron depleted media. Changes in fluorescence were analyzed as described in the Materials and Methods. Results for the sodB promoter fusions are displayed in Panel 1A, and results for the pfr promoter fusions are displayed in Panel 1B. For both A and B, solid lines indicate the plasmid in WT H. pylori G27 grown in iron replete conditions, dotted lines indicate the plasmid in WT bacteria grown in iron deplete conditions, and dashed lines indicate the plasmid in Δfur bacteria grown in iron replete conditions. Fluorescence is measured in relative units, and the data are representative of multiple independent flow analyses.

doi:10.1371/journal.pone.0005369.g001
A. Exponential Phase

|         | WT G27 | WT 26695 | Δfur G27 | Δfur 26695 |
|---------|--------|----------|----------|------------|
| sodB    | 0.9    | 0.8      | 1.3      | 0.9        |
| pfr     | 0.3    | 0.06     | 0.9      | 1.3        |
| amiE    | 9.0    | 2.5      | 3.5      | 1.0        |

B. Stationary Phase

|         | WT G27 | WT 26695 | Δfur G27 | Δfur 26695 |
|---------|--------|----------|----------|------------|
| sodB    | 1.5    | 1.6      | 2.7      | 1.3        |
| pfr     | 0.25   | 0.04     | 2.0      | 2.9        |
| amiE    | 12.5   | 12.4     | 16.4     | 1.3        |

Figure 2. Direct Comparison of sodB Regulation in H. pylori Strains G27 and 26695. WT and Δfur strains of G27 and 26695 were grown to exponential (A) and stationary (B) phase in iron replete and iron-limited (growth) media (60 μM dpp). After growth overnight, one-half of the exponential phase iron replete culture was removed for RNA isolation. 200 μM dpp (final concentration) was added to create an iron-depletion shock condition to the remaining half of the iron replete cultures, and those cultures were grown for an additional hour prior to RNA isolation. The same procedure was applied the following day to the iron replete, stationary phase culture. After overnight growth, one-half of the iron-limited growth culture was removed for RNA isolation in exponential phase while the remaining half was allowed to grow into stationary phase, and RNA was isolated the following day. RNase Protection Assays (RPAs) were performed on RNA isolated from these strains using sodB, pfr, and amiE riboprobes. Data for Exponential phase cultures are shown in Panel A, and data for Stationary phase cultures are shown in Panel B. Fold-changes are indicated below each pair and were calculated by comparing either the relative amount of protected riboprobe in the iron-depletion shock environment (S) or the relative amount of protected riboprobe in the iron limited growth environment (G) to the iron replete lane (N). These data are representative of multiple independent experiments.

Analysis of the role an amino acid (AA) difference in Fur plays in sodB regulation

Given the difference in sodB regulation between the two strains, we reasoned that either a difference in Fur or a difference in sodB between the two strains was likely to be responsible for the change. We therefore aligned the predicted Fur amino acid sequence from G27 and 26695 to determine if there were any obvious differences between the two strains that might account for the differences in sodB regulation. As shown in Fig. 3A, the last AA was found to differ between the strains. In G27 AA 150 is a Tyr while in 26695 it is a Cys. To determine if this AA difference had any role in Fur-dependent regulation of sodB, a “Fur swap” strain was created, which completely replaced the G27 fur coding sequence with the coding sequence from 26695. RPAs were then conducted on RNA harvested from WT G27, WT 26695, and the “Fur swap” strain. Results are shown in Fig. 4. In order to show the reproducibility of the data, RPA data is represented in a graphical format. In this manner the fold change for each strain and biological repeat is displayed as a point on the graph. Additionally, the median fold change is depicted as a bar to allow for easy comparison between the strains. Because the decrease in sodB expression in 26695 is most pronounced in exponential phase, only results of RPAs performed using exponential phase RNA are shown.
26695 Fur in G27 (the “Fur swap” strain) did not restore apo-Fur sodB regulation in G27 under either iron-limited growth or iron-depletion shock conditions (Fig. 4A and data not shown). However, apo-Fur regulation of *pfr* was as expected in all three strains (Fig. 4B and data not shown) [8]. Because the trends of the growth data for both the *sodB* and *pfr* RPA data were similar to the shock, the growth data has not been shown.

While the AA difference in Fur was apparently not responsible for the difference in *sodB* regulation, we wondered if the levels of *fur* expression were similar between the different strains. To test this, RPAs were performed on RNA isolated from all three strains using a *fur* riboprobe. The basal level of *fur* expression in each strain was then compared to that of WT 26695 as shown in Fig. 4C. While the level of *fur* expression in the G27 strain was slightly higher than in 26695, no substantial differences in *fur* expression were found between the strains.

As Fur has been shown to be autoregulatory, repressing its own expression in the presence of iron [25,26], we also compared Fur autoregulation between G27, 26695, and the “Fur swap” strain. *fur* RPAs were performed on RNA isolated from each strain, and an increase in *fur* expression was seen for G27, 26695, and the “Fur swap” strain under iron-depletion shock conditions while little to no increase was seen under iron-limited growth conditions (Fig. 4D and data not shown). This data shows that Fur autoregulation is consistent in each strain and further supports the notion that the AA difference in Fur is not responsible for the difference in *sodB* regulation between G27 and 26695.

**RPA determination of the role the −5 bp of the *sodB* promoter plays in *sodB* regulation**

Since the difference in *sodB* regulation between G27 and 26695 appeared not to be related to the difference in the Fur coding sequence, we next considered that there might be differences in the *sodB* promoter between the strains that could account for the discrepancy in regulation. Therefore, we sequenced the *sodB* promoter from G27 and compared it to the known *sodB* promoter sequence from 26695 [14]. As shown in Fig. 3B, a single base change was evident in the Fur Box. Previous DNA Footprint analysis showed that Fur protects a region that extends from −5 bp to −47 bp within the *sodB* promoter [1]. At the −5 bp, G27 encodes a C while 26695 encodes an A. To determine if this nucleotide difference was important for *sodB* regulation, a “−5 bp swap” strain was engineered such that the G27 promoter would encode an A at the −5 bp position. RPAs were then conducted on RNA isolated from the “−5 bp swap” strain along with WT G27 and WT 26695, and results are shown in Fig. 5. While *sodB* expression remained unchanged in G27 under iron depletion shock conditions, a two-fold decrease in *sodB* expression was observed in the “−5 bp swap” strain (Fig. 5A). The difference in fold decrease between G27 and the “−5 bp swap” was statistically significant with a p-value of 0.0066, as was the difference between G27 and 26695 with a p-value of 0.0001. While the fold decrease in *sodB* expression in the “−5 bp swap” strain under iron-limited growth conditions did not reach 2-fold, it was consistently higher than its G27 counterpart (data not shown), apo-Fur regulation of *pfr* in each of these strains was similar and as expected [8] (Fig. 5B and data not shown). These data suggest that a single nucleotide difference within the *sodB* promoter is at least partially responsible for the difference in regulation of this gene between G27 and 26695.

**Comparison of *sodB* regulation in various strains of *H. pylori***

Given the differences in *sodB* regulation in G27 and 26695, we wondered if other *H. pylori* strains exhibited apo-Fur regulation similar to G27 or 26695. Therefore, we also examined J99 and HPAG1. Analysis of the *sodB* promoter sequences of these two additional strains showed that at the −5 bp HPAG1 encodes a C similar to G27, and J99 encodes a G that is different from all other strains (Fig. 3B). Given that the A at the −5 bp seems to be crucial for apo-Fur regulation of *sodB*, we predicted that these strains would show Fur regulation of *sodB* similar to what was seen with G27. To test this, RPAs were performed on RNA isolated from J99 and HPAG1. As shown in Fig. 4, neither J99 nor HPAG1 displays the expected decrease in *sodB* expression [1]; both behave similarly to G27 (Fig. 4A). However, *pfr* expression (Fig. 4B), basal levels of *fur* expression (Fig. 4C), and fur autoregulation (Fig. 4D) are preserved in J99 and HPAG1. Taken together, these data

---

**A. Fur Alignment**

![Fur Alignment](image)

**B. sodB Promoter Alignment**

![sodB Promoter Alignment](image)

---

Figure 3. Alignments of Fur and of the *sodB* promoters. Panel A contains the alignment of the predicted Fur amino acid sequences of G27 and 26695. As indicated by an arrow, amino acid 150 is different between the two strains. Panel B contains the *sodB* promoter alignment from G27, 26695, J99, and HPAG1 with essential promoter elements indicated. The predicted Fur Box ranges from bases −5 to −47 and is indicated by the dashed box [1]. The −5 bp difference between the strains is indicated with an arrow in Panel B. Alignments for both panels were constructed using MultAlin software [37].

doi:10.1371/journal.pone.0005369.g003
suggest that natural polymorphisms found at the $-5$ bp of the sodB promoter in different \textit{H. pylori} strains affect the regulation of sodB by apo-Fur.

\textbf{In vitro binding of Fur to different sodB promoters}

Given that the $-5$ bp in the sodB promoter appears to play some role in the apo-Fur regulation of sodB, we next investigated the direct interaction of apo-Fur with the various sodB promoters. To assay the binding of apo-Fur, we performed Electrophoretic Mobility Shift Assays (EMSAs) and competition studies for each sodB promoter (WT G27, "-5 bp swap," and WT 26695) using purified Fur under apo reaction conditions \cite{1}. As shown in Fig. 6, Fur binds to and retards the mobility of each of the three sodB promoters, but not the control \textit{rpoB} promoter. Moreover, the addition of homologous unlabeled sodB promoter DNA was able to compete for Fur binding with each sodB promoter thus confirming specific interaction between Fur and the sodB promoters (Fig. 6).

Because apo-Fur was able to bind to and shift each of the three sodB promoter fragments and because our expression data showed that the $-5$ bp was important for regulation, we reasoned that the various promoter fragments should show differences in their affinity for Fur. To test this, each labeled sodB promoter fragment was competed with varying concentrations of its own (homologous) unlabeled promoter fragment as well as with each of the other unlabeled promoter fragments. The success of the competition was then measured by quantitating the percent of unbound probe resulting from each competition reaction such that $P_{32}D_{P} < P_{32}D_{P}$, where $P$ = Fur, $D_{32}$ = labeled DNA, and $D$ = cold competitor. As shown in Fig. 7, the various promoter fragments showed differences in affinity such that 26695 $>$ $-5$ bp $>$ G27. In all cases, the 26695 and $-5$ bp promoter were better able to compete for Fur binding as the largest percentages of unbound labeled promoter fragment are observed with these two promoters in comparison to the WT G27 sodB promoter. Taken together with the expression data, these data indicate that the $-5$ bp is important for Fur interaction at the sodB promoter.

\textbf{Discussion}

Given how pleomorphic \textit{H. pylori} is, it is not surprising that genes may be regulated differently in different strains. Indeed, there have been several instances of this reported in the literature in recent years involving acid-response and CodRS \cite{27}, vacA regulation \cite{28}, virulence gene regulation \textit{in vivo} \cite{29}, and \textit{cagA} and \textit{vacA} expression in response to salt \cite{30}. In addition, a single nucleotide polymorphism upstream of the Fur-box was found to alter Fur regulation of \textit{IrgA} in two different strains of \textit{E. coli} \cite{31} indicating that there may be more to Fur regulation in other organisms than just binding at the recognition sequence. This study adds to that body of knowledge and is the first to explore the differences in Fur regulation among different strains of \textit{H. pylori}. 

---

\textbf{Figure 4. Strain specific differences in sodB regulation.} Various \textit{H. pylori} strains were grown to exponential phase as described in the Materials and Methods, and RNA was isolated from iron replete and iron-depleted shock conditions. RPAs were performed using sodB, pfr, and fur riboprobes and results are displayed in Panels A, B, and D, respectively. Basal levels of fur expression relative to the level of expression in 26695 are depicted in Panel C. Fold decrease in expression for sodB and pfr, fold increase for fur, and relative levels of basal fur expression are plotted as single points for each strain with squares, diamonds, triangles, and circles. Each shape represents a biologically independent set of RNA. Median fold change is represented as a bar for each strain. The dotted-dashed line represents the 2-fold significance cut-off in Panels A, B, and D. In Panel A only, the triangles represent the average of two technical repeats on that independent set of RNA. doi:10.1371/journal.pone.0005369.g004
apo-Fur regulation remains a unique form of Fur regulation found only in *H. pylori*. Additionally, our understanding of this type of regulation is currently limited as only two apo-Fur repressed genes, *sodB* [1] and *pfr* [8], have been characterized. Here we present evidence that *H. pylori* shows strain specific differences in *sodB* apo-regulation that are partially controlled by a natural polymorphism found at the −5 bp of the *sodB* promoter.

Alteration of this single nucleotide in the G27 promoter to resemble the residue found in 26695 resulted in alteration of G27 *sodB* regulation that mimicked regulation seen in 26695. Based on this observation, we accurately predicted that two other commonly used strains of *H. pylori*, J99 and HPAG1, would show altered *sodB* regulation since they each encode a different nucleotide at the −5 position within the *sodB* promoter.

The importance of the −5 bp within the *sodB* promoter is further supported by our EMSA competition data. At low concentrations of competitor DNA, the “−5 bp swap” promoter is able to bind to apo-Fur with an affinity similar to WT 26695 while WT G27 exhibits weaker binding. At higher concentrations of competitor, the affinity of the “−5 bp swap” promoter for apo-Fur is still greater than WT G27 but slightly less than WT 26695. Thus, it appears that strain specific regulation of *sodB* is due to differences in the affinity of Fur for the various promoters and that natural polymorphisms at the −5 bp are largely responsible for this differential regulation.

The significance of the *sodB* polymorphism in *H. pylori* fitness, especially *in vivo*, is currently unclear. However, the affinity of apo-Fur for the *sodB* promoter in 26695 was reported to be relatively weak (*K_d* = 260 nM) [1], and based upon our competition data it is likely even weaker in G27. As Ernst, et al. suggested, a weak affinity between apo-Fur and the *sodB* promoter makes physiological sense, as SodB is the only defense *H. pylori* has against superoxide radical damage [1,10]. Therefore, it would be ill-advised to repress *sodB* under conditions where any iron is still available.

Figure 5. Role of the −5 bp in *sodB* regulation. WT G27, WT 26695, and the “−5 bp swap” strain were grown as described in the Materials and Methods, and RNA was isolated under iron replete and iron-depletion shock conditions. RPAs were performed on RNA isolated from 4 biologically independent experiments using *sodB* and *pfr* riboprobes. Data from *sodB* RPAs are presented in Panel A, and data from *pfr* RPAs are presented in Panel B. Each square, diamond, triangle, and circle represent the average fold decrease calculated from three technical repeats with each independent set of RNA for each strain and growth condition combination. Median fold decrease is represented as a bar for each combination, and the dotted-dashed line represents the 2-fold significance cut-off. *p-value of 0.0001. †p-value of 0.006.

doi:10.1371/journal.pone.0005369.g005
available, since iron catalyzed oxidative damage could still be possible [1]. In keeping with this, some strains of *H. pylori* may have evolved to either inactivate apop-Fur regulation of sodB, or to weaken repression by decreasing the Fur/sodB binding affinity. Also of note, as shown in Fig. 2, in the absence of Fur, iron chelation results in slight increases in sodB (and pfr) perhaps suggesting the presence of additional regulatory proteins that ensure proper expression of this critical factor.

Furthermore, it is interesting to speculate that strains, which possess sequences similar to 26695, might actually show decreased *in vivo* fitness due to decreased expression of sodB in the iron limited environment of the stomach. Analysis of the sodB promoter sequence in the efficient gerbil colonizing strain B128 (isolate 7.13) [32] revealed that B128, similar to G27, encodes a C at the −5 bp (data not shown). Therefore, studies could potentially be designed with this strain that would allow for the determination of whether direct apop-Fur regulation of sodB provides a competitive advantage to *H. pylori* in *vivo*.

Currently, little is understood about the sequences recognized by *H. pylori* Fur that dictate binding of the protein at target promoters. This is true of both iron-bound and apop forms of Fur. In *E. coli*, Fur binding has been shown to involve recognition of a well-conserved consensus sequence called a Fur Box. This Fur Box consists of two

![Figure 6. Fur binding to the sodB promoters. EMSAs were performed by incubating various concentrations of purified Fur with radiolabeled fragments of the WT G27, “−5 bp swap,” and WT 26695 sodB promoters as well as the negative control promoter, rpoB, as detailed in the Materials and Methods. In the first four lanes, the Fur concentrations are indicated by the triangle from highest to lowest and range from 1.07 μg/mL to 0.026 μg/mL. A no protein control for each promoter is found in the fifth lanes. The last lane shows the 100X cold (unlabeled) competition control for each promoter fragment, which were each performed with the highest concentration of Fur (1.07 μg/mL). Fur exhibits specific interaction with each of the sodB promoters, and no interaction with the rpoB promoter except for very little nonspecific binding at the highest Fur concentration. These data are representative of multiple independent EMSA experiments. doi:10.1371/journal.pone.0005369.g006](image)

![Figure 7. Competitive Binding Studies. To assess the relative affinity of Fur for each of the sodB promoter fragments (WT G27, “−5 bp swap,” and WT 26695), Fur was incubated with each radiolabeled promoter and 5X, 10X, or 25X the amount of homologous or heterologous unlabeled sodB promoter fragments as described in the Materials and Methods. For each labeled promoter, lane one contains a no competition control. Lanes two to four, five to seven, and eight to ten contain the competition EMSAs with unlabeled WT G27, “−5 bp swap,” and WT 26695 sodB fragments, respectively. The percent of labeled promoter that is outcompeted and remains unbound in each lane is given below each image. These data are representative of multiple independent experiments. doi:10.1371/journal.pone.0005369.g007](image)
9 bp inverted repeat sequences separated by a single A nucleotide to create a 19 bp palindromic sequence as follows: GATAATGATGATATATGC [33]. This sequence can also be interpreted as a series of three hexamer repeats of NATATA[T]AT [34]. However, in H. pylori this E. coli Fur Box is not conserved, and consensus is currently ill-defined. For iron-bound Fur regulation, the binding sequence occurs in AT-rich regions in the target promoter (as repeats of AAAT [8,24,25,35,36]). There is no defined consensus sequence for apo-Fur binding given that the two promoters of the known apo-Fur regulated genes, pfr and sodB, share only minimal homology [1,8]. In an organism that has about 60% A/T residues in its genome, a Fur Box consensus sequence that is comprised of mainly these two nucleotides does not seem to be an ideal approach for Fur regulation. Rather, in H. pylori it is perhaps more plausible that both iron-bound and apo-Fur recognize unique DNA structures that are required for proper regulation of their target genes. The work presented here is the first to define a residue that is important for both iron-bound and apo-Fur regulation with the hope that continued exploration of Fur regulation will provide greater understanding into the complexity of gene regulation in this important human pathogen.

Acknowledgments

We thank R. Peek for providing B129 isolate 7.13, C. Olsen for assistance with data presentation, R. Maier for providing the pET21A vector, A. vanVliet and J. Stoof for assistance with the EMSA protocol, E. Maynard and T. Dunn for assistance with protein purification, and members of the Merrell lab for help with cell harvesting.

Author Contributions

Conceived and designed the experiments: BMC HG DSM. Performed the experiments: BMC HG RPN DSM. Analyzed the data: BMC JMW SLJM DSM. Contributed reagents/materials/analysis tools: ALW JMW SLJM. Wrote the paper: BMC SLJM DSM.

References

1. Ernst FD, Homuth G, Stoof J, Madir U, Waidner B, et al. (2003) Iron-responsive regulation of the Helicobacter pylori iron-reductase superoxide dismutase SodB is mediated by Fur. J Bacteriol 185: 3679–3692.
2. Dunn BB, Cohen H, Blaser MJ (1997) Helicobacter pylori. Clin Microbiol Rev 10: 720–741.
3. Blaser MJ (1999) Helicobacter pylori and the pathogenesis of gastrointestinal inflammation. J Infect Dis 161: 626–633.
4. van Amsterdam K, van Vliet AH, Kusters JG, van der Ende A (2006) Of iron and pH homeostasis intersect at the level of Fur regulation in the gastric pathogen Helicobacter pylori. Mol Microbiol 59: 131–156.
5. Berreswill S, Lichte F, Vey T, Fassbinder F, Kist M (1998) Cloning and characterization of the fur gene from Helicobacter pylori. FEMS Microbiol Lett 159: 279–284.
6. Bury-Mone S, Thibere JM, Conresras M, Maioutourn A, Labigne A, et al. (2004) Responsiveness to acidity via metal ion regulators mediates virulence in the gastric pathogen Helicobacter pylori. Mol Microbiol 53: 623–638.
7. Gancz H, Censini S, Morrell DS (2006) Iron and pH homeostasis intersect at the level of Fur regulation in the gastric pathogen Helicobacter pylori. Infect Immun 74: 602–614.
8. Delany I, Spohn G, Rappuoli R, Scarlato V (2001) The Fur repressor controls transcription of iron-activated and -repressed genes in Helicobacter pylori. Mol Microbiol 42: 1297–1309.
9. Spiegelhalder C, Gerstenecker B, Kersten A, Schiltz E, Kist M (1993) Purification of the 128-kDa immunodominant antigen of Helicobacter pylori. J Bacteriol 175: 169–174.
10. Wang G, Conover RC, Ohl C, Alamuri P, Johnson MK, et al. (2005) characterization of the 128-kDa immunodominant antigen of Helicobacter pylori. J Bacteriol 187: 193–200.
11. Wang G, Conover RC, O’Shea AA, Alumari P, Johnson MK, et al. (2005) Oxidative stress defense mechanisms to counter iron-promoted DNA damage in Helicobacter pylori. Free Radic Res 39: 1183–1191.
12. Guttridge JM, Maith L, Poyer L (1990) Superoxide dismutase and Fenton chemistry. Reaction of ferric-EDTA complex and ferric-haemoglobin complex with hydrogen peroxide without the apparent formation of iron(II). Biochem J 269: 169–174.
13. Covacci A, Censi S, Bugnoli M, Petracca R, Burroni D, et al. (1993) Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytoadhesion and leukocyte ingestion. Proc Natl Acad Sci U S A 90: 5791–5795.
14. Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, et al. (1997) The complete genome sequence of the gastric pathogen Helicobacter pylori. Nature 388: 539–547.
15. Eaton KA, Morgan DR, Krakowska S (1989) CagA/patB/sodB expression in human cells. Infect Immun 57: 1119–1123.
16. Rashid RA, Tarr PI, Moseley SL (2006) Expression of the sodB gene of Helicobacter pylori strain: BMC HG RPGN DSM. Analyzed the data: BMC JMW SLJM. Wrote the paper: BMC SLJM DSM.