Supporting Information

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SI Results

Purification of *Campylobacter jejuni* Ferric Uptake Regulator Protein and Generation of Polyclonal Anti-Ferric Uptake Regulator Antibodies Suitable for ChIP Experiments. We expressed and purified the *C. jejuni* (Cj) ferric uptake regulator (Fur) protein to generate specific anti-CjFur antibodies. The obtained protein was determined to be ~95% pure, and was used to generate polyclonal rabbit antibodies against *C. jejuni* Fur.

Western Blot Analysis Confirms anti-CjFur Specificity. Western blotting demonstrated that the anti-Fur antibodies were specifically targeted against the Fur protein, with a complete loss in reactivity in the Δ*fur* deletion mutant (Fig. S5). Additional testing demonstrated that these antibodies do not react with the Fur homolog PerR that is also present in *C. jejuni*.

SI Materials and Methods

Bacterial Strains and Growth Conditions. The bacterial strains and plasmids used in this study are listed in Dataset S1. All *C. jejuni* strains were grown at 37 °C under microaerophilic conditions [83% N_2, 4% H_2, 8% O_2, and 5% CO_2 (vol/vol)] in Mueller–Hinton (MH) broth, MEMα medium supplemented with 10 mM pyruvate and 40 μM $FeSO_4$, or on MH agar plates. Media were supplemented as required with chloramphenicol (20 μg/mL) or kanamycin (10 μg/mL). Plasmids were constructed and propagated in *Escherichia coli* DH5α. All *E. coli* strains were grown at 37 °C in Luria–Bertani (LB) broth or on LB agar plates. When relevant, ampicillin, chloramphenicol, or kanamycin was added to the *E. coli* LB medium at a concentration of 100 μg/mL, 10 μg/mL, or 50 μg/mL, respectively.

Construction of *C. jejuni* Fur Expression Vectors. The *fur* gene was PCR-amplified from *C. jejuni* TGH9011 with Pfx (Invitrogen) using the primers furSE and furAS, which contain a BsaI restriction site on their ends (Dataset S1). TGH9011 Fur contains a single amino acid substitution (T42I) compared with NCTC11168 Fur. This site is not conserved across Fur proteins (Fig. 2), and would not be expected to influence Fur binding. The PCR products were purified using a Qiagen PCR purification kit and then cut with BsaI and ligated into a pASK-IBA7 vector to create pASK-Fur. This vector was used to transform *E. coli* DH5α by electroporation. Transformed cells were selected by plating on LB agar plates supplemented with 100 μg/mL ampicillin. Selected colonies were grown in LB broth supplemented with 100 μg/mL ampicillin to midlog phase and aliquoted for storage at −80 °C. The resulting plasmids were sequenced to ensure the presence of unaltered *C. jejuni fur* sequence. A strain containing the correct Fur sequence was designated as AS1130 and subsequently used to produce CjFur protein for antibody production. A different construct was used to purify the Fur protein for crystallographic studies to avoid the presence of purification tags (see below). Briefly, the *C. jejuni fur* sequence from pASK-Fur was PCR-amplified and cloned in-frame with a PCR sequence containing a Strep tag followed by the sequence for SMT3 (SUMO). The resulting Strep-SMT3-CjFur cassette was then digested with NdeI and XhoI, cloned into pET3d, and subsequently transformed into Rosetta cells.

Expression and Purification of *C. jejuni* Fur Protein for Antibody Production. CjFur protein was expressed and purified from AS1130 according to the manufacturer's instructions (IBA; Strep-tag). Briefly, cells were grown to midlog phase in LB broth at 37 °C with shaking at 200 rpm, and protein expression was then induced by adding anhydrotetracycline to a final concentration of 200 ng/mL. The cells were incubated for 3 h at 37 °C with shaking and then pelleted by centrifugation at 6,000 × *g* for 10 min. The cells were resuspended in 100 mM Tris·HCl (pH 8.0) and 150 mM NaCl buffer (wash buffer) with the addition of Complete Mini Protease Inhibitor (Roche), incubated with 5 μg/mL DNase I and 1 mg/mL lysozyme for 30 min at 4 °C, and subsequently lysed by sonication. The lysates were applied to a Strep-Tactin Sepharose column (IBA), washed five times with wash buffer, and eluted with wash buffer supplemented with 2.5 mM desthiobiotin. Fractions were analyzed by SDS/PAGE to determine the presence of purified protein. CjFur eluted as a doublet on SDS/PAGE gels, as has been previously observed for Fur-family proteins (1, 2). Both bands were excised from SDS/PAGE gels and sequenced using LC-MS/MS (Ottawa Institute of Systems Biology) and found to be *C. jejuni* Fur. The obtained protein was determined to be ~95% pure and was used to generate polyclonal rabbit antibodies against *C. jejuni* Fur.

Anti-CjFur Antibody Production. Anti-CjFur antibodies were produced by Invitrogen by inoculating rabbits with 1 mg of purified Fur protein. Bleeds were taken at 4, 8, and 10 wk after inoculation. The bleeds were pooled and further purified with an affinity column constructed from purified CjFur protein. An ELISA was used to confirm a specific response to *C. jejuni* Fur, and also indicated that there was no preexisting reactivity to CjFur protein before inoculation.

Western Blot Analysis. *C. jejuni* NCTC11168 strain and Δ*fur* mutant were grown to midlog phase in biphasic MH cultures. The cells were pelleted by centrifugation at 6,000 × *g* for 10 min, resuspended in PBS buffer with Complete Mini Protease Inhibitor (Roche), and lysed by sonication. Protein content was quantified using a Quant-It Protein Assay Kit (Invitrogen). Ten micrograms of each cell lysate along with 100 ng of purified CjFur protein was run on a 12.5% SDS/PAGE gel for 60 min at 120 V and subsequently transferred to a PVDF membrane at 100 V for 60 min. The membranes were blocked overnight at 4 °C in 5% skim milk powder. The membranes were then probed with 1:1,000 anti-CjFur antibody for 1 h followed by washing and incubation with 1:10,000 anti-rabbit IgG antibody conjugated with HRP. Blots were incubated with Pierce ECL (Fisher) chemiluminescent substrate according to the manufacturer's instructions and visualized using X-ray film.

Immunoprecipitation of DNA–Fur Complexes. *C. jejuni* NCTC11168 and Δ*fur* strains were grown to midlog phase in MEM-α medium supplemented with 10 mM sodium pyruvate and 40 μM $FeSO_4$ under microaerophilic conditions. Previous work had demonstrated that these iron-replete conditions would result in repression of known Fur-regulated targets and thus ensure that Fur protein would be bound to its target sequences and allow for comparison with our previously obtained transcriptomic data. However, as cells were harvested during midlog phase, it is unlikely that all Fur proteins would be metallated, and a fraction of the Fur would be present in the apo form. All cross-linking steps were performed under the same microaerophilic conditions. Briefly, the cells were treated with 1.1% (vol/vol) formaldehyde for 10 min to cross-link protein–DNA complexes. The cross-linking was stopped with the addition of 0.125 M glycine for 15 min. The cells were then pelleted at 6,000 × *g* for 10 min and washed with cold RIPA buffer (50 mM Tris·HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 0.1% Na deoxycholate, and 0.1% Nonidet P-40). Bacterial pellets

were resuspended in 2 mL RIPA buffer, and 200 μL of a 40 mg/mL bacterial protease inhibitor was added (Sigma). Cells were lysed and DNA was fragmented by sonication. Cell debris was removed by centrifugation for 7 min at 11,000 × *g*, and samples were run on agarose gels to determine the degree of DNA fragmentation. Sonication times were determined empirically to generate an average DNA fragment size of ~500 bp. Fragmented DNA lysates were preincubated with protein G agarose (Sigma) for 3 h at 4 °C. The agarose was removed by centrifugation at 13,000 × *g* for 1 min and the cleared lysates were incubated with 10 μg of anti-Fur antibody overnight at 4 °C. Protein G agarose was added for 4 h at 4 °C, after which the agarose–antibody–chromatin complex was harvested by centrifugation at 13,000 × *g* for 1 min and washed five times with cold RIPA buffer, followed by resuspension in 500 μL of 50 mM Tris·HCl (pH 7.5), 10 mM EDTA, and 1% SDS. Elution of DNA was performed by incubating this suspension for 20 min at 65 °C, followed by centrifugation at 13,000 × *g* to remove the agarose, and cross-linking was reverted by incubation of the supernatant for 10 h at 65 °C. Finally, the DNA was purified and washed five times with 450 μL ddH_2O using a μM-YM30 filter (Millipore) and then dried with a SpeedVac vacufuge and resuspended in 55 μL H_2O. Chromatin immunoprecipitation with the Δ*fur* mutant constituted our negative control.

Amplification and dUTP Labeling of Immunoprecipitated DNA. Immunoprecipitated DNA was amplified following the protocol from Agilent (Mammalian ChIp-on-chip version 10.0). Briefly, the DNA ends were blunted with T4 DNA polymerase (Invitrogen), and linkers (Dataset S1) were ligated on the ends by T4 DNA ligase (New England BioLabs). The resulting DNA pool was PCR-amplified using JW102 as a primer. An additional round of PCR amplification was used to generate sufficient quantities of DNA for microarray analysis. Once sufficient quantities of DNA had been generated, the DNA was labeled with aminoallyl-dUTP with the BioPrime Labeling System (Invitrogen) for 3 h at 37 °C and subsequently purified using a Qiagen PCR purification kit per the manufacturer's instructions.

Probe Labeling, Microarray Hybridization, and Analysis. The aminoallyl-labeled DNA was concentrated under vacuum using a SpeedVac and resuspended to 10 μL with 0.1 M sodium carbonate buffer (pH 9). The aminoallyl-labeled cDNA was coupled to indocarbocyanine or indodicarbocyanine dye (GE Healthcare) as previously described. The fluorescently labeled cDNA was purified using Qiaquick PCR spin columns according to the manufacturer's instructions (Qiagen). Differentially labeled immunoprecipitated DNA derived from *C. jejuni* NCTC11168 or Δ*fur* was pooled, dried under vacuum, and dissolved in 36 μL of hybridization buffer [5× SSC buffer (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7), 0.1% SDS, 25% formamide, and 25 μg salmon sperm DNA]. The labeled cDNA pool was hybridized to a *C. jejuni* NCTC11168 microarray. The construction and validation of the *C. jejuni* microarrays have been previously described (3). The hybridization was performed under a coverslip using a prehybridized microarray slide (slides that have been incubated at 42 °C for 45 min in 5× SSC buffer, 0.1% SDS, 25% formamide, and 1% BSA). Following an 18-h incubation at 42 °C in a humidified chamber (Arrayit), the microarray slides were washed consecutively in 2× SSC/0.1% SDS for 5 min, 0.1× SSC/0.1% SDS for 10 min at room temperature, and four times in 0.1× SSC for 1 min at room temperature. Finally, the slides were washed with distilled water, dried by centrifugation, and scanned with a laser-activated confocal scanner (ScanArray Gx; PerkinElmer) at 10-μm resolution.

ChIP Microarray Data Analysis. The signal intensities for each spot were collected using ScanArray Express software (PerkinElmer). Spots within regions of printing or hybridization anomalies were excluded from the analysis. The raw fluorescence intensity values were background-subtracted. Spots with background-subtracted values below three times the SD of the local background in both channels were also excluded from further analysis. Subsequently, the background-subtracted fluorescence intensity in each wavelength [channel 1 for indocarbocyanine (Cy3) and channel 2 for indodicarbocyanine (Cy5)] was normalized using MIDAS software (available from TIGR; http://www.tigr.org/software) and by applying an iterative log-mean centering algorithm. Microarray data from four independent biological experiments were collected, yielding 21 measurements per gene (because the probe for each gene is printed three times on each microarray). Genes with less than four non-zero measurements were excluded from further analysis. Finally, the ratio of the channels 2:1 was converted to $\log_2$, and the data were statistically analyzed with the empirical Bayes method as previously described (4–6). Chromosomal regions exhibiting reproducible and statistically significant enrichment were classified as CjFur binding sites. The locations of the Fur-binding regions were manually refined by taking into consideration gene annotation, operonic structure, and transcriptional direction. Given that our ChIP protocol generated fragments of 0.5–1.0 kb in size and ~62% of *C. jejuni* genes are <1 kb in length, we were not able confidently to distinguish between intergenic and intragenic binding. Genes were considered to be enriched if they had a fold enrichment [ratio (Fur-IP from *C. jejuni* wild type):(Fur-IP from Δ*fur*) ≥1.5 (NCTC/Δ*fur*)] with a Bayesian *P* value ≤0.001.

Quantitative PCR Confirmation of ChIP-on-Chip Targets. Selected genes were confirmed for enrichment using quantitative (q)PCR. DNA templates were prepared as described for the microarray analysis, except that the immunoprecipitated DNA was not amplified or labeled. Both immunoprecipitated and input genomic DNA from NCTC11168 and Δ*fur* cultures were analyzed to ensure equal loading and template quality. Quantitative PCR reactions were done using Taq polymerase in a final volume of 50 μL. The reaction conditions were as follows: 1× PCR buffer, 11.4 mM $MgCl_2$, 0.06% Triton X-100 (Fisher), 4 nM ROX reference dye, 0.16× SYBR Green, 8 U/mL Taq, 0.2 μM each primer, and either 1 ng immunoprecipitated DNA or 100 ng input genomic DNA. All reagents were obtained from Invitrogen unless otherwise noted. Fold enrichment was calculated as previously described (6) using the $\Delta\Delta C_T$ method, with primers for *ilvC* gene acting as an endogenous control. Similar fold changes were obtained when using *ahpC* as an alternate endogenous control.

COG Functional Enrichment. CjFur ChIP-enriched genes were categorized according to their annotated clusters of orthologous groups (COG) category. COG categories that contained at least three members were selected for further analysis to determine whether any categories were overrepresented within the Fur regulon. Functional enrichment of COG categories in Fur-regulated genes was determined by performing a one-tailed Fisher's exact test with a Bonferroni correction, and $P < 0.05$ was considered significant.

Determining Fur Transcriptional Regulation. Previous work has used transcriptional approaches to define the Fur regulon by comparing the transcriptome of wild-type NCTC11168 to an isogenic *fur* deletion mutant in the presence and absence of iron. This allowed for the identification of genes that were differentially expressed in a Fur- and iron-dependent manner (holo-Fur regulation) and a Fur- and iron-independent manner (apo-Fur regulation). The analysis of these data was repeated as previously described (5) and used to assign identified Fur ChIP targets as holo-Fur–activated/repressed, apo-Fur–activated/repressed, and unknown (Table S1). The regulation of several genes was also assayed using RT-qPCR (see below for details).

Bioinformatic Analysis of Potential Fur Binding Sites. The promoter regions of CjFur ChIP-enriched trancriptional units were analyzed for the existence of potential CjFur binding sites by the MEME suite of analysis tools (http://meme.nbcr.net). Transcriptional units were analyzed as a whole and also as subgroups based on their potential mode of CjFur regulation (apo-CjFur activation/repression, holo-CjFur activation/repression). Regions consisting of 250 bp upstream of the predicted start codon (both with/without gene overlap) of each transcriptional unit were retrieved from the Regulatory Sequence Analysis Tools server (http://rsat.ulb.ac.be) and analyzed for potential regulatory motifs using MEME. In the case of transcriptional units in operonic structure, we also tested for consensus sequences present in the promoter region of the predicted lead transcript. Consensus sequence logos from identified motifs were constructed using WebLogo (http://weblogo.berkeley.edu). The transcriptional units that were used to generate the holo-CjFur repression motif consisted of *katA*, *chuA*, *cj1613c*, *trxB*, *cj0177*, *cj0176c*, *ceuB*, *cj1384c*, *cj1627c*, *cj0263*, and *cj0262c*. The holo-CjFur activation motif was generated using *cj0145*, *cj1097*, *cj1349c*, *cj1476c*, *cj1588c*, *cj1650*, *flaB*, *flaG*, *napA*, and *rrc* (5, 6).

RT-qPCR Analysis of Fur ChIP Targets. Total RNA was extracted from *C. jejuni* NCTC11168 and Δ*fur* cultures as previously described (5). Briefly, cells were grown to midlog phase in MEM-α medium with 10 mM sodium pyruvate and either with or without 40 μM $FeSO_4$ supplementation under microaerophilic conditions. Cells were harvested by adding 10% cold RNA, stop solution was added to the cultures (10% buffer-saturated phenol in absolute ethanol), and cells were pelleted by centrifugation at 6,000 × *g*. Cells were resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and RNA was extracted using a hot phenol/chloroform protocol. The extracts were washed five times in 80% ethanol after overnight RNA precipitation, and RNA pellets were resuspended in TE buffer. RNA extracts were treated with DNase I to remove contaminating genomic DNA. Treated samples were purified using the RNeasy Kit from Qiagen and tested for the absence of DNA using PCR. RT-qPCR was performed on an Applied Biosystems 7300 thermocycler using the QuantiTect SYBR Green RT-PCR Kit from Qiagen as described previously (6). Gene fold changes were determined according to the $\Delta\Delta C_T$ method using *slyD* as an endogenous control. The primer sets used for *dsbB*, *cj1345c*, and *rrc* are listed in Dataset S1; the primers for *katA* and *slyD* have been described elsewhere (6, 7). Genes were considered differentially expressed if their expression was altered by >1.5-fold with $P < 0.05$. Genes that had positive fold changes in NCTC11168 compared with the Δ*fur* mutant are activated; negative fold changes are indicative of repression. Genes differentially expressed under iron-limited conditions were considered as being apo-Fur–regulated, and genes differentially expressed under iron-replete conditions were considered as being holo-Fur–regulated.

CjFur Purification and Crystallization. CjFur was overexpressed in Rosetta cells as a Strep-SUMO fusion protein following induction with 0.1 mM isopropyl β-D-1-thiogalactopyranoside for 3 h at 37 °C. Rosetta cells were centrifuged at 3,000 rpm (BECKMAN J-6B centrifuge, USA) for 30 min and resuspended in PBS buffer (pH 7.5). Cells were lysed by sonication and centrifuged at 31,000 × *g* for 30 min, and the supernatant was clarified by filtration. The filtered solution was applied to Strep-TACTIN (Novagen) beads and the Strep-TACTIN was washed with 15 column volumes (CV) of PBS. The fusion protein was eluted according to the manufacturer's instructions, and the Strep-SUMO tag was removed using the SUMO-specific protease ULP1 by a 16-h incubation at 4 °C. CjFur was further purified by applying the proteins onto SP Sepharose (GE Healthcare) equilibrated in 20 mM citrate (pH 6.0) and 5 mM β-mercaptoethanol (buffer A). CjFur was eluted with 10 CV of buffer A supplemented with 300 mM NaCl and further purified by size-exclusion chromatography (Superdex 75). The protein eluted as a single peak at an apparent molecular mass of ~34 kDa, corresponding to a dimer. CjFur fractions were pooled and concentrated to 10 mg/mL. Plate-shaped crystals were grown in 200 mM $MnSO_4$ and 10–20% PEG3350 at 4 °C. Crystals were harvested in the mother liquor supplemented with 20% ethylene glycol and flash-frozen in liquid nitrogen.

Inductively Coupled Plasma Mass Spectrometry Metal Analysis. Inductively coupled plasma mass spectrometry (ICPMS) was performed at the Quantitative Bioelemental Imaging Center, Northwestern University, using a Thermo Fisher X Series II ICP-MS system. Each MS spectrum was recorded in triplicate using two serial dilutions (1/20 and 1/100) of purified CjFur. Table S2 shows that the CjFur preparation is predominantly found in a complex with Zn^{2+} with trace amounts of Cu^{2+}.

Data Collection and Crystal Structure Determination. The datasets were collected at the Life Sciences Collaborative Access Team beamline at the Advanced Photon Source. CjFur's structure was determined by single-wavelength anomalous diffraction at the zinc peak wavelength (Table S3). The reflections were processed and scaled using HKL2000 (8), and four zinc atoms were identified and refined using the SHELX C/D programs (9). Phases were calculated using SHELX-E (9), and the ARP/wARP program (http://www.embl-hamburg.de/ARP/) was used to generate the initial model. Two chains (referred therein as protomers A and B) were traced to near completion (140 amino acids for chain A and 120 residues for chain B). This initial protein structure was then used as a search model for molecular replacement using Phaser (http://www.ccp4.ac.uk/). The missing residues were modeled in the calculated phases using Coot (10), and the structure was further refined using Refmac (11). The quality of the model was assessed using MolProbity (12, 13), and analysis of protein backbone ϕ/ψ angles, calculated using MolProbity, indicates that 97.8% of nonglycine residues are found in the favored regions of the Ramachandran plot.

Gel-Mobility Assays. ***Holo-Fur.*** Forward and reverse Cy5-labeled primers corresponding to a 40-bp DNA fragment of the *katA* promoter region were purchased from Eurofins MWG Operon. Oligonucleotides were annealed by incubation at 95 °C for 10 min and slowly cooled down to room temperature. For the gel-shift assays, 0, 5, 50, and 100 nM purified recombinant CjFur was incubated with 50 μM $MnCl_2$ for 30 min on ice in binding buffer (20 mM bis-Tris borate, pH 7.4, 50 mM KCl, 3 mM $MgCl_2$, 5% glycerol, 0.1% Triton X-100, and 50 μM $MnCl_2$). Cy5 (1 nM)-labeled DNA fragment and 1 μg of poly(dI-dC) were then added to the protein and incubated for 30 min on ice. Samples were run for 50 min at 100 V on a 6% nondenaturing polyacrylamide gel (19:1) at 4 °C. Gels were freshly prepared with 100 mM bis-Tris borate (pH 7.4) and 100 μM $MnCl_2$ (buffer A) and prerun for 30 min at 150 V at 4 °C using buffer A as running buffer.

Apo-Fur. The promoter regions upstream of the *rrc* and *Cj1345c* operons (~200 bp) were PCR-amplified from *C. jejuni* using the following primer pairs with the forward primers labeled with Cy5: Cjrrc_For/Cjrrc_Rev and Cj1345c_For/Cj1345c_Rev (Dataset S1) (Eurofins MWG Operon). Approximately 2.5 nM Cy5-labeled DNA fragment was incubated with increasing amounts of purified recombinant CjFur (0, 50, 100, 200, and 1000 nM) for 40 min on ice in a binding buffer devoid of $MnCl_2$. Samples were run for 60 min at 100 V on a 5% nondenaturing polyacrylamide gel (19:1) at 4 °C.

All gel-shift assays were scanned using the Typhoon scanner (GE Healthcare; Typhoon Trio) and analyzed using ImageQuant TL software (www.gelifesciences.com/iqtl).

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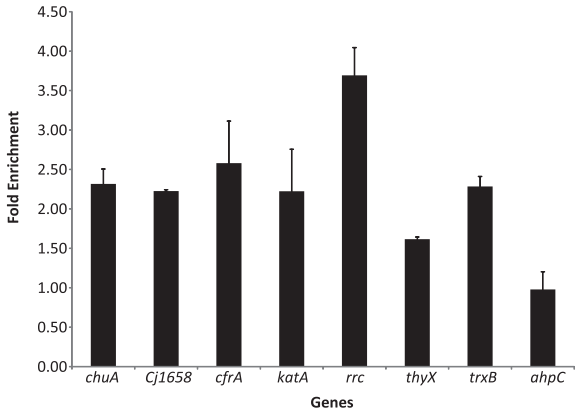

Fig. S1. Quantitative PCR validation of selected Fur ChIP targets. Selected genes were chosen for qPCR validation of CjFur enrichment. The fold enrichment was determined by the $\Delta\Delta C_T$ method compared with a negative control (*ilvC*). The CjFur-enriched genes were also seen to be enriched by qPCR analysis (*chuA*, *Cj1658*, *katA*, *rrc*, *thyX*, *trxB*), whereas an additional negative control (*ahpC*) did not show enrichment.

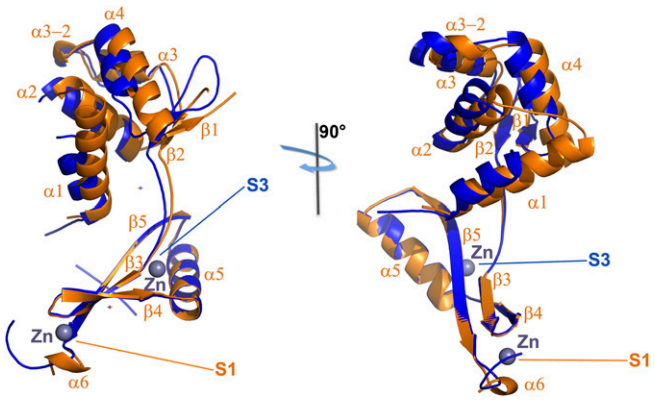

Fig. S2. CjFur dimer is asymmetric. Shown are two orthogonal views of CjFur aligned protomers in which β-strands, α-helices, and metal binding sites are rendered as in Fig. S3.

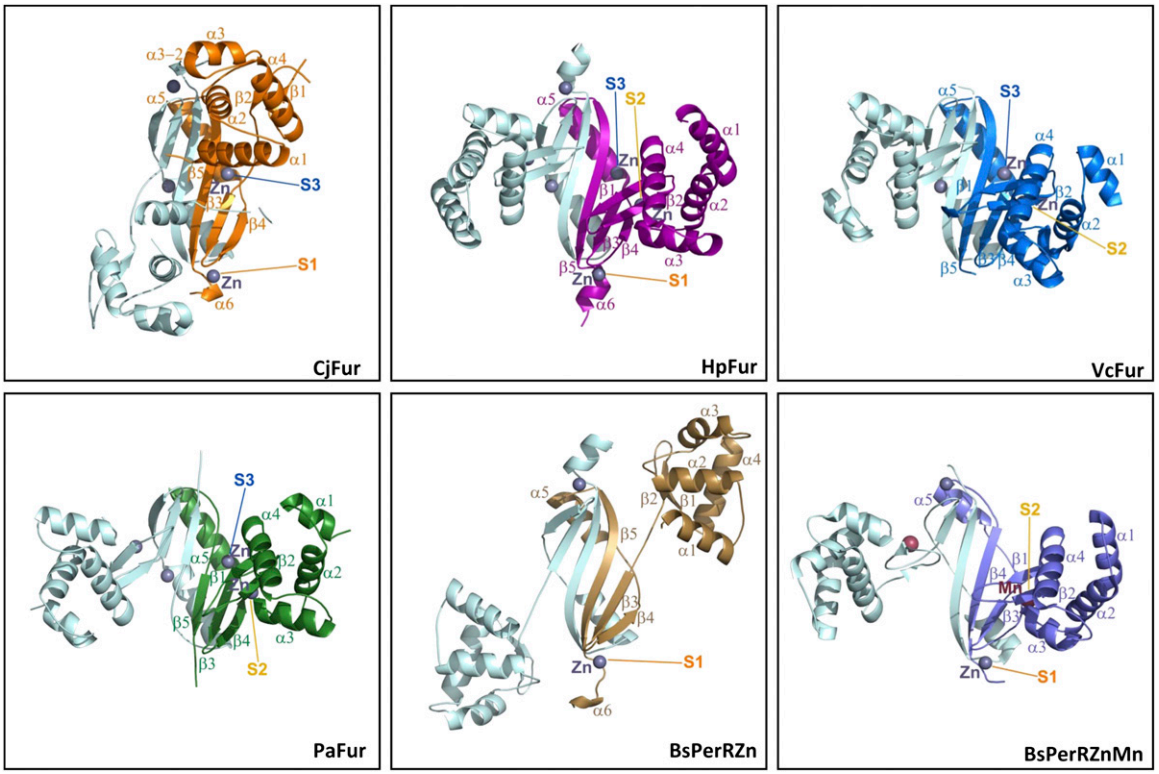

Fig. S3. CjFur DNA-binding domain adopts an atypical conformation. Overall structure of Fur and Fur-like proteins in which protomer A is highlighted in orange (CjFur), purple [*Helicobacter pylori* (Hp)Fur; 2XIG], blue [*Vibrio cholerae* (Vc)Fur; 2W57], green [*Pseudomonas aeruginosa* (Pa)Fur; 1MZB], brown [*Bacillus subtilis* (Bs)PerRZn; 2FE3], and violet (BsPerRZnMn; 3F8N). β-Sheets, α-helices, and metal binding sites of each protomer A are labeled accordingly. To facilitate the analysis, the dimerization domains are shown in the same orientation. All structures were obtained from the RCSB PDB structure bank (http://www.rcsb.org/pdb/).

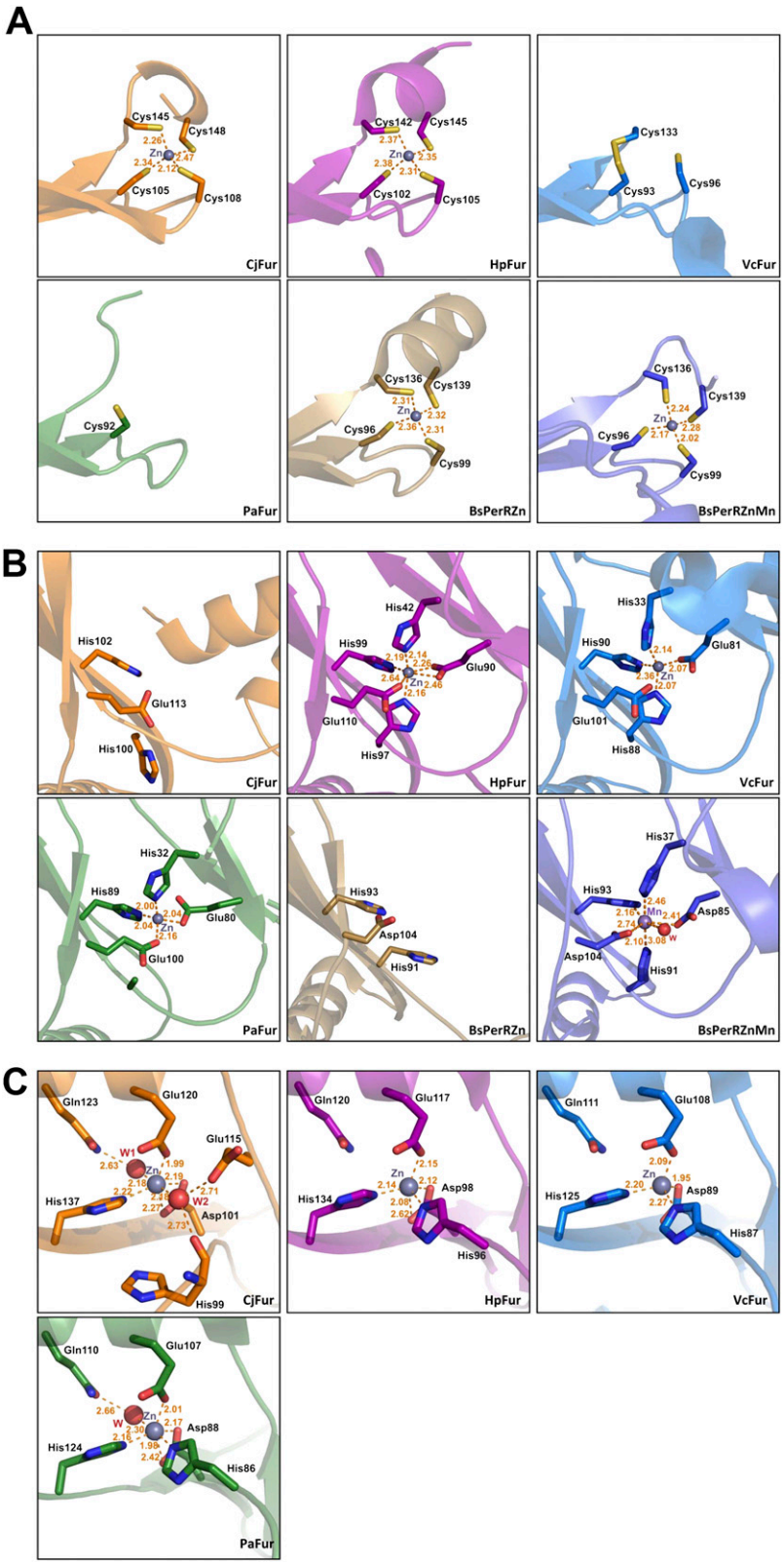

Fig. S4. CjFur metal coordination diverges from other Fur and Fur-like proteins. S1 (*A*), S2 (*B*), and S3 (*C*) metal binding sites of CjFur, HpFur (2XIG), VcFur (2W57), PaFur (1MZB), BsPerRZn (2FE3), and manganese-bound BsPerRZn (3F8N). Carbon atoms are rendered as in Fig. S3, whereas zinc and manganese atoms are highlighted in gray and purple, respectively. Hydrogen bonds and water molecules are depicted as orange dashed lines and red spheres, respectively. All structures were obtained from the RCSB PDB structure bank (http://www.rcsb.org/pdb/).

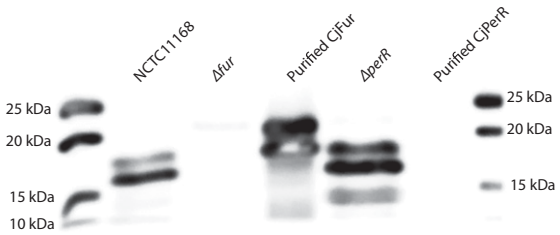

Fig. S5. Confirmation of anti-CjFur antibody specificity. The specificity of the anti-CjFur antibody was determined by Western blot analysis. Either 10 μg of protein lysate or 100 ng of purified protein was separated by SDS/PAGE. Anti-Fur antibodies reacted with protein in the NCTC11168 and Δ*perR* mutant *C. jejuni* strains, as well as the purified CjFur protein. This reactivity was lost in the Δ*fur* mutant, and the anti-Fur antibodies also did not react with purified CjPerR.

Other Supporting Information Files

Table S1 (XLSX)
Table S2 (DOCX)
Table S3 (DOCX)
Dataset S1 (XLS)