Supporting Information

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SI Materials and Methods

Bacterial Strains. Strains and plasmids are listed in Table S3. *Vibrio cholerae* El Tor biotype strain C6706 and a spontaneous *lacZ*− derivative of C6706 were used as parental (WT) strains. Antibiotic concentrations used were streptomycin (Sm; 100 μg/mL), kanamycin (Kan; 50 μg/mL), and chloramphenicol (Cm; 2.5 μg/mL for C6706 and 10 μg/mL for *Escherichia coli* DH5α λpir. LB contained 10 g/L of tryptone (Bacto), 5 g/L of yeast extract (Bacto), and 5 g/L of NaCl, and was supplemented with 16 g/L of agar (Bacto) for growth on plates. Arabinose was used at 0.1% for ChIP assays. $FeSO_4$ (Sigma) was used for iron supplementation at a final concentration of 40 μM. $NiCl_2$ was added at 1.0 μM.

DNA Manipulations. For p*fur*, *V. cholerae* ferric uptake regulator (vcFur) was amplified from chromosomal DNA and cloned into plasmid pBAD18 carrying a C-terminal 3× V5 epitope tag after digestion with KpnI and SalI. All cloned products were sequence verified. Primers are listed in Table S4.

ChIP. Fifty milliliters of exponentially growing culture in LB + 40 μM $FeSO_4$ were induced with 0.1% arabinose for 30 min at 37° C. Formaldehyde was added to a final concentration of 1% and incubated at RT for 20 min with occasional swirling. Crosslinking was quenched by adding glycine to 0.5 M. Cell pellets were washed in 1× TBS and resuspended in lysis buffer [10 mM Tris (pH 8.0), 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% deoxycholate (DOC), 0.5% *N*-lauroylsarcosine] plus protease inhibitor mixture (Sigma) and 1 mg/mL lysozyme and were incubated at 37° C for 30 min. The cells were sonicated 1× for 30 s with a needle sonicator, and unlysed debris was pelleted by centrifugation. The lysate was sonicated for 20 min with a 10-s on/10-s off cycle (Mixsonix). A sample was taken as a sequencing input control. Following clarification by centrifugation, 1/10 volume of 10% Triton X-100 in lysis buffer was added to each sample followed by 100 μL of Dynal-Protein G beads coated with anti-V5 monoclonal antibody (Sigma), and samples were incubated overnight with rotation. The beads were washed 5× with RIPA buffer [50 mM Hepes (pH7.5), 500 mM LiCl, 1 mM EDTA, 1% Nonidet P-40, 0.7% DOC] and then 1× in Tris-EDTA pH 8.0 plus 50 mM NaCl and were resuspended in 100 μL elution buffer [50 mM Tris-HCl (pH 7.5), 10 mM EDTA, 1% SDS]. Samples were incubated at 65° C for 30 min, and the beads were pelleted by centrifugation. Supernatants were incubated at 65° C overnight to reverse crosslinks. Samples were incubated with 8 μL of 10-mg/mL RNase A for 2 h at 37° C and then with 4 μL of 20 mg/mL proteinase K at 55° C for 2 h and were purified with Qiagen MinElute Reaction Cleanup Kit and quantitated with Pico green kit (Invitrogen). Experiments were performed in triplicate.

Next-Generation Sample Preparation and Sequencing. One to three nanograms of ChIP or input DNA was processed for sequencing by the addition of a polyA tail as described by Helicos protocols (http://www.helicosbio.com/). Samples were sequenced using the HeliScope Single Molecule Sequencer at the Molecular Biology Core Facility in the Dana-Farber Cancer Institute, Boston, MA.

Sequence Data Processing. Data from each sequencing run were processed using Helisphere openware to generate FASTA format sequence reads. The sequence reads were aligned to the *V. cholerae* N16961 genome using CLC genomic workbench software. CLC genomic workbench ChIP coupled with next-generation sequencing (ChIP-seq) software was used to compare control and experiment alignments to identify peak enrichment. We applied a 100-bp sliding window and a false-discovery rate cutoff of 0.001% to identify peaks. Peaks called in at least two of the three experimental samples were scored as real vcFur ChIP peaks.

ChIP peak length is dictated by DNA shear size. Our average DNA shear size from sonication is 250 bp. Because shearing is random, vcFur can be located anywhere along the 250-bp average length, including the extreme termini. Because sequencing of ChIP DNA occurs from both ends, the ChIP peak is ~500 bp. Downstream analysis defines where in this peak the actual binding site is located. Our analysis compiled all full-length vcFur ChIP peaks and then searched for a conserved binding motif. Motif analysis was performed with MEME suite motif-based sequence analysis tools (1).

Quantitative PCR. For ChIP-seq peak validation, relative-abundance quantitative PCR (qPCR) was performed with Kapa Biosystems Fast SYBR green mix using 16S and 5S rDNA targets as controls. Relative target levels were calculated using the $\Delta\Delta C_t$ method, with normalization of ChIP targets to16S rDNA signal (2). For gene expression analysis, absolute-expression qPCR was performed with the Applied Systems RNA-C_t one-step system (3). Briefly, the number of molecules of each transcript in total RNA was determined by comparing C_t values with a standard curve specific for each target gene. *fur*::Tn/WT expression was determined by comparing the number of molecules of target in each sample. Primers are listed in Table S4. All samples were taken at the logarithmic growth phase. The growth rates for *fur*::Tn and WT strains were the same under our conditions.

vcFur Purification and Band-Shift Assays. 6×-His C-terminal–tagged vcFur expressed in BL21 *E. coli* was purified by affinity chromatography with a Co^{2+}NTA resin (Thermo) in 50 mM Hepes (pH 7.4) and 300 mM NaCl. Apo-vcFur was prepared as described (4). ^{32}P-labeled DNA duplexes (1 nM final concentration) were mixed with increasing amounts of vcFur (0–20 nM final concentration) in 10 mM Tris (pH 7.5), 20 mM KCl, 5% glycerol, 0.1 mg/mL BSA, 150 μM $ZnSO_4$, 7 μm β-mercaptoethanol in a final volume of 10 μL and were incubated on ice for 10 min. Sucrose-loading dye (10 μL) was added, and the mixture was separated on a 6% Tris-Borate gel and imaged with film. Experiments were repeated four times with similar results. The ratio of shifted-band to input-band intensity from all four experiments was determined by densitometry. The ratios from Box 1 and Box 2 were compared with determine the fold increase in affinity of vcFur for Box 1.

Northern Blots. RNA was prepared from logarithmic cultures in triplicate using Ambion RiboPure Kit. Total RNA (10–15 μg) was separated on a 6% Tris/Borate/EDTA-urea gel and transferred to Hybond N membrane. After crosslinking and before hybridization, membranes were incubated with 100 pmol of ^{32}P-labeled probe. Washed membranes were exposed to film overnight. Probes are listed in Table S4.

1. Bailey TL, Elkan C (1994) Fitting a mixture model by expectation maximization to discover motifs in biopolymers. *Proc Int Conf Intell Syst Mol Biol* 2:28–36.
2. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. 25:402–408.
3. Yoder-Himes DR, et al. (2009) Mapping the Burkholderia cenocepacia niche response via high-throughput sequencing. *Proc Natl Acad Sci USA* 106:3976–3981.
4. Sheikh MA, Taylor GL (2009) Crystal structure of the Vibrio cholerae ferric uptake regulator (Fur) reveals insights into metal co-ordination. *Mol Microbiol* 72:1208–1220.

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)
Table S3 (DOCX)
Table S4 (DOCX)