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A Proteomic Analysis Reveals Differential Regulation of the $\sigma^S$-Dependent yciGFE(katN) Locus by YncC and H-NS in Salmonella and Escherichia coli K-12

Mélanie Beraud§¶, Annie Kolb§¶, Véronique Montei§¶, Jacques D’Alayer¶, and Françoise Norel§¶‡

The stationary phase sigma factor $\sigma^S$ (RpoS) controls a regulon required for general stress resistance of the closely related enterobacteria Salmonella and Escherichia coli. The $\sigma^S$-dependent yncC gene encodes a putative DNA binding regulatory protein. Application of the surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF) ProteinChip technology for proteome profiling of wild-type and mutant strains of Salmonella enterica serovar Typhimurium revealed potential protein targets for YncC regulation, which were identified by mass spectrometry, and subsequently validated. These proteins are encoded by the $\sigma^S$-dependent operon yciGFE(katN) and regulation of their expression by YncC operates at the transcriptional level, as demonstrated by gene fusion analyses and by in vitro transcription and DNase I footprinting experiments with purified YncC. The yciGFE genes are present (without katN) in E. coli K-12 but are poorly expressed, compared with the situation in Salmonella. We report that the yciGFE(katN) locus is silenced by the histone-like protein H-NS in both species, but that $\sigma^S$ efficiently relieves silencing in Salmonella but not in E. coli K-12. In Salmonella, YncC acts in concert with $\sigma^S$ to activate transcription at the yciG promoter (pycG). When overproduced, YncC also activated $\sigma^S$-dependent transcription at pycG in E. coli K-12, but solely by countering the negative effect of H-NS. Our results indicate that differences between Salmonella and E. coli K-12, in the architecture of cis-acting regulatory sequences upstream of pycG, contribute to the differential regulation of the yciGFE(katN) genes by H-NS and YncC in these two enterobacteria. In E. coli, this locus is subject to gene rearrangements and also likely to horizontal gene transfer, consistent with its repression by the xenogeneic silencer H-NS. Molecular & Cellular Proteomics 9: 2601–2616, 2010.

In eubacteria, transcription depends on a multisubunit RNA polymerase (RNAP) consisting of a catalytically active core enzyme (E) with a subunit structure $\alpha_2\beta\beta’\omega$, that associates with any one of several $\sigma$ factors to form different holozyme (E$\sigma$) species. The $\sigma$ subunit is required for specific promoter binding, and different $\sigma$ factors direct RNAP to different classes of promoters, thereby modulating gene expression patterns (1). The RNA polymerase holozyme containing the $\sigma^{70}$ subunit is responsible for the transcription of most genes during exponential growth (1). When cells enter stationary phase or are under specific stress conditions during exponential growth, $\sigma^S$, encoded by the rpoS gene, becomes more abundant, associates with the core enzyme, and directs the transcription of genes essential for the general stress response (1–3). In the closely related Enterobacteria Salmonella and Escherichia coli, $\sigma^S$ is required for stationary phase survival, stress resistance, and biofilm formation. It is also involved in the virulence of Salmonella enterica serovar Typhimurium (S. Typhimurium) (4).

Transcriptome analyses in S. Typhimurium and E. coli K-12 have shown that rpoS controls more than 300 genes, 40% of which are of unknown function (3, 5, 6). A large fraction of $\sigma^S$-controlled genes encode putative regulators and signal transducing factors, suggesting that $\sigma^S$ controls a complex network with regulatory cascades and signal input at levels downstream of $\sigma^S$ itself. We previously used a bank of S. Typhimurium mutants to identify $\sigma^S$-regulated genes (7). One of these genes, the yncC gene (7), encoded a putative DNA binding protein of the GntR/FadR family of bacterial regulators (8–10). To further investigate the function of the yncC gene, we decided to characterize the proteome of the Salmonella yncC mutant by the surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF$^\dagger$) ProteinChip technology.

The SELDI-TOF method is based on the selective protein retention on a solid-phase chromatographic chip surface and successive analysis by simple laser desorption/ionization mass spectrometry (11). Because of its high-throughput nature and experimental simplicity, this technology has been widely used for protein profiling of tissues and biomarker discovery (11) and unpublished work from our laboratory re-
Regulation of yciGFEkatN in Salmonella and E. coli

revealed the efficiency of this in characterizing the RpoS-dependent proteome of Salmonella. In the present study, potential protein targets for YncC regulation in Salmonella were revealed by SELDI-TOF, identified and subsequently validated by in vivo and in vitro analyses. These proteins are encoded by the Salmonella yciGFEkatN operon controlled by σ^5 (12). The binding of YncC upstream of the yciG promoter and its effects on σ^5-dependent transcription were investigated.

During the course of this work, it was reported that mcbbR, the ortholog of yncC in E. coli K-12, represses the transcription of the ybiM gene, which prevents overproduction of colanic acid and subsequent inhibition of biofilm formation (13). We report here that ybiM is not present in Salmonella, prompting investigation of the possibility that these two orthologs perform different regulatory functions in E. coli K-12 and Salmonella by studying activation of yciGFE gene expression by YncC/McbR in E. coli K-12. The results reveal differential regulation of the yciGFE(katN) locus by YncC and H-NS (the Histone-like Nucleoid Structuring protein, 14–16) in these two closely related bacteria.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, and Growth Conditions—Strains and plasmids are listed in Table I. Bacteriophage P22HT105/1int was used to transfer mutations between Salmonella strains by transduction (26). Green plates, for screening for P22-infected cells or lysogens, were prepared as described previously (27). Bacteriophage P1 transduction (28) was used to construct mutants in E. coli K-12 using mutants available from the KEIO collection (20) (Table I). Strains were routinely cultured in Luria Bertani medium (LB) (17). Antibiotics were used at the following concentrations: ampicillin, 100 μg/ml; carbenicillin, 100 μg/ml; chloramphenicol, 15 μg/ml for the chromosomal resistance gene and 30 μg/ml for the plasmid resistance gene; kanamycin, 50 μg/ml; and tetracycline 20 μg/ml.

DNA Manipulations and Sequence Analysis—Standard molecular biology techniques were used (17). Oligonucleotides were obtained from Sigma-Aldrich (France). DNA sequencing was performed by Beckman Coulter Genomics (France). DNA and amino acid sequence analyses were conducted using the BLAST programs at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/), the Genome Center at Washington University (http://genome.wustl.edu/genomes), and the Sanger Institute (http://www.sanger.ac.uk/Projects/Salmonella/). Other Web sites for sequence analyses were http://www.ncbi.nlm.nih.gov/kegg and http://enterix.cbcb.umd.edu/. Physico-chemical parameters of protein sequences were predicted using ProtParam (ExPASy Web site).

Construction of Plasmids—The nucleotide sequence of a PCR-amplified yncC gene (STM1588) from S. Typhimurium ATCC14028 revealed that it is identical to that in S. Typhimurium LT2 (http://genomoid.wustl.edu/projects/bacterial/styphimurium/) and to that in the recently published genome sequence of ATCC14028 (GenBank CP001363) (29). A 1.3 kb BamHI fragment carrying the kanamycin resistance cartridge from pUC4K was ligated into the BamHI restriction site in pACYC184, resulting in pACK. pACKynC was constructed using primers YncC-E1 and YncC-E2 (Table II) to amplify the promoter-less yncC gene from ATCC14028 total DNA by PCR. EcoRI restriction sites were incorporated at its 5’ and 3’ ends. Following digestion with EcoRI, the fragment was inserted into the EcoRI site of pACK to give pACKynC (the yncC and cat genes are in the same orientation and the yncC gene is likely transcribed from the cat promoter). The nucleotide sequence of the yncC insert in pACKynC was verified by DNA sequencing. pynC^HIS, which expresses an N-terminal His6-fusion to the yncC gene product under the control of the pQE30 IPTG-inducible promoter, was constructed as follows. Primers YncC-H3 and YncC-H5 (Table II) were used to amplify the yncC gene from ATCC14028 total DNA by PCR. BamHI and HindIII restriction sites were incorporated at its 5’ and 3’ ends, respectively. Following digestion with BamHI and HindIII, the PCR-amplified fragment was ligated into the BamHI and HindIII sites of pQE30. The nucleotide sequence of the yncC insert in pynC^HIS was verified. Construction of plasmid for in vitro transcription was a follows. The E. coli yciG fragment (extending from –2277 to +66 relative to the transcription start) was synthesized from primers M91 and M92 and the S. Typhimurium yciG fragment (~184 to ~48) from primers M47 and M48bis. The fragments were cleaved by EcoRI and BamHI and inserted into the pJCD01 vector cleaved by EcoRI and BamHI, leading to plasmids pJCDycG and pJCDykN.

Construction of the ΔyncC Δhns and Δyci Mutants of S. Typhimurium—Chromosomal deletions in the yncC, hns and yciGFEkatN loci of Salmonella ATCC14028 were generated using PCR-generated linear DNA fragments and the λRed recombination method as described by Datsenko and Wanner (30). Briefly, 63–66 nt primers with 43–46 nt homology with the gene of interest on the 5’ end of the primer and 20 nt homology with the FLP recognition target flanked antibiotic resistance cassette of plasmid pKD3 at the 3’ end (sequences given in 30) were used. The primer pairs, YncC-P1 and YncC-P2, Hns-P1 and Hns-P2, YciG-P1 and KatN-P2 (Table II) were used for disruption of the yncC, hns, and yciGFEkatN operon, respectively. ATCC14028 containing the plasmid pKD46, which carries the λ recombination genes gam, bet, and exo under control of the araBAD promoter (30) was grown overnight at 30 °C, diluted in LB carbenicillin containing l-arabinose 1 mM and grown to an OD600 of 0.5. Electrocompetent cells were prepared, transformed with the PCR-generated linear fragments and plated on LB containing chloramphenicol (15 μg/ml) and incubated at 37 °C. The resulting colonies were characterized using a combination of PCR reactions using locus-specific primers and common test primers (30). Finally, isogenic strains were characterized using a combination of PCR reactions using locus-specific primers and common test primers (30). PCR assays were then used to ensure integration of the plasmids in the correct location and to determine the presence of multiple plasmid integrants (using common test primers, such as those described in (31)). Locus-specific flanking primers were also used to amplify junction fragments that were subsequently analyzed by DNA sequencing. Isogenic strains were constructed by P1 mediated transduction of the mutations into the appropriate strains. When required, the chloramphenicol resistance cassette was eliminated using a temperature-sensitive helper plasmid pCP20, which encodes the FLP recombinase (30). Because the hns mutants were very sick and might accumulate compensatory mutations, they were constructed freshly for each experiment.

Construction of a Chromosomal yciE-lacZ Transcriptional Fusion in E. coli K-12—A single copy yciE-lacZ transcriptional fusion was constructed from mutant MC4100yciE using conditional plasmids containing promoter-less lacZ genes and the FLP recognition target site as described (31). PCR assays were then used to ensure integration of the plasmids in the correct location and to determine the presence of multiple plasmid integrants (using common test primers, such as those described in (31)). Locus-specific flanking primers were also used to amplify junction fragments that were subsequently analyzed by DNA sequencing. Isogenic strains were constructed by P1 mediated transduction of the mutations into the appropriate strains.

Protein Profiling by SELDI-TOF-MS—Bacteria were grown in LB for 18 h at 37 °C. Cells were harvested and cell pellets obtained from 100 ml of culture were resuspended in 20 ml phosphate buffer 50 mm pH 7 and disrupted in a Cell Disruptor (Constant Systems, Daventry, UK).

The abbreviations used are: LB, Luria Bertani; SELDI-TOF-MS, surface-enhanced laser desorption/ionization -time of flight-mass spectrometry; s/n, signal to noise.
### Table I

**Bacterial strains and plasmids used in this study**

| Strain or plasmid                  | Characteristics                                      | Source or reference |
|-----------------------------------|------------------------------------------------------|---------------------|
| **Escherichia coli**              |                                                      |                     |
| JM109                             | recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi Δ(lac-proAB) F'[(traD36 proAB lacI lacZ M15)] | 17                  |
| MG1655                            | F- Δ(iBV) rfb-50 rph-1                               | 18                  |
| MC4100                            | Δ(lacIPOZYA)X74, galU, galK, strA-R, Δ(luxA-lexZ)    | Laboratory stock    |
| MC1061                            | araD139 Δ(luxA-lexZ)-767 Δ(lacIPOZY)X74 rpsL         | 17                  |
| popH118                           | F- araD139 Δ(argF-lac)-U169 rpsL150 relA1            | 19                  |
| JW5437                            | ΔpoS::Km                                             | 20                  |
| JW1445                            | ΔnciC::Km                                            | 20                  |
| JW1249                            | ΔyciE::Km                                            | 20                  |
| JW1250                            | ΔyciF::Km                                            | 20                  |
| MG1665yciE                       | MG1655 ΔyciE::Km                                     |                     |
| MG1665yciF                       | MG1655 ΔyciF::Km                                     |                     |
| MG1665hns                        | MG1655 Δhns-118                                      |                     |
| MG1665yciEhns                    | MG1655 ΔyciE::Km                                     | Δhns-118            |
| MG1665yciEhns                    | MG1655 ΔyciF::Km                                     | Δhns-118            |
| MC4100yciE                       | MC4100 ΔyciE::Km                                     |                     |
| MC4100hns                        | MC4100 Δhns-118                                      |                     |
| MC1061hns                        | MC1061 Δhns-118                                      |                     |
| MC4100poS                        | MC4100 ΔpoS::Km                                      |                     |
| MC4100yciE-lacZ                   | MC4100 yciE-lacZ                                     |                     |
| MC4100mcbR yciE-lacZ              | MC4100 yncC yciE-lacZ                                |                     |
| MC4100poS yciE-lacZ               | MC4100 ΔpoS yciE-lacZ                                |                     |
| MC4100hns yciE-lacZ               | MC4100 Δhns-118                                      |                     |
| MC4100poShns yciE-lacZ            | MC4100 ΔpoS yciE-lacZ                                | Δhns-118            |
| **Salmonella serovar Typhimurium** |                                                      |                     |
| ATCC14028                         | Wild-type ATCC14028 yncC::Tn5B21-12F-12               | ATCC<sup>a</sup>    |
| ATCC-F12                          | ATCC14028 yncC::Tn5B21-12F-1                         | 7, 21               |
| ATCC-F1                           | ATCC14028 yncC::Tn5B21-12F-1                         | 7, 21               |
| ATCC katN-lacZ                    | ATCC14028 katN::Tn5B21-12G-12                        |                      |
| ATCCkatpoS                       | ATCC14028 ΔpoS::Km                                    | 22                  |
| ATCCsyncC                       | ATCC14028 ΔyncC::Km                                   |                     |
| ATCCΔyci                         | ATCC14028 ΔyciF::Km                                   |                     |
| ATCCkatns                        | ATCC14028 Δhns::Km                                    |                     |
| ATCCsyncC katN-lacZ               | ATCC14028 ΔyncC::Km katn::Tn5B21-12G-12               |                     |
| ATCCkatpoS katN-lacZ              | ATCC14028 ΔpoS::Km katn::Tn5B21-12G-12                | 21                  |
| ATCCkatns katN-lacZ               | ATCC14028 Δhns::Km katn::Tn5B21-12G-12                |                     |
| ATCCkatnpoS katN-lacZ             | ATCC14028 ΔpoS::Km katn::Tn5B21-12G-12                |                     |
| ATCCkatnsyncC katN-lacZ           | ATCC14028 ΔyncC::Km katn::Tn5B21-12G-12               |                     |
| ATCC2922K                         | ATCC2922K ΔSTM12G2:Km                                 | 21                  |
| ATCC2922KpoS                      | ATCC2922K ΔpoS::Km                                    | 21                  |
| ATCC2922Kkatns                    | ATCC2922K Δhns::Km                                    | 21                  |
| ATCC2922KpoS katN-lacZ            | ATCC2922K ΔpoS::Km katn::Tn5B21-12G-12                | 21                  |
| ATCC2922Kkatns katN-lacZ          | ATCC2922K Δhns::Km katn::Tn5B21-12G-12                |                     |
| ATCC2922KkatnpoS katN-lacZ        | ATCC2922K ΔpoS::Km katn::Tn5B21-12G-12                |                     |
| ATCC2922KkatnsyncC katN-lacZ      | ATCC2922K ΔyncC::Km katn::Tn5B21-12G-12               |                     |
| ATCC2922KkatnpoS syncC katN-lacZ  | ATCC2922K ΔpoS::Km katn::Tn5B21-12G-12                |                     |
| ATCC2922KkatnsyncC katN-lacZ      | ATCC2922K ΔyncC::Km katn::Tn5B21-12G-12               |                     |
The cell debris were removed by centrifugation. Protein concentrations in the supernatants were determined using the DC Protein Assay kit (Bio-Rad) and adjusted to a concentration of 1 mg/mL. Negatively charged proteins were captured on the array using 100 μl of the resin Q Ceramic HYPERD F (BioSepra-Pall, Cergy St Christophe, France) can be used for protein purification, was employed to capture protein fragments upstream of the rmb gene (yciG). The cell extracts were stored at −20 °C. Spectra were externally calibrated with cytochrome C (bovine) (43,240.0 Da), Conalbumine (77,490.0 Da), and IgG bovine (147,300.0 Da). The baseline was established using a smoothing of three points and a width of five times the expected peak width and spectral intensities were normalized by total ion current. Consistent peak sets of similar mass across the spectra were used to prepare the ProteinChip array. A strong anion exchange ProteinChip array (Q10, Bio-Rad), for which the complementary resin Q Ceramic HYPERD F (BioSepra-Pall, Cergy St Christophe, France) can be used for protein purification, was employed to capture protein fragments upstream of the rmb gene (yciG).

### Table I—continued

| Strain or plasmid | Characteristics | Source or reference
|-------------------|-----------------|---------------------|
| pACYC184         | Cloning vector, Cm<sup>R</sup>, Tet<sup>R</sup> | 23 |
| pACK             | pACYC184::Km, Cm<sup>R</sup>, Km<sup>R</sup> | 19 |
| pUCsyncC         | pACK with the promoterless yncC gene cloned into the cat gene (yncC is transcribed from the cat promoter), Km<sup>R</sup> | 25 |
| pUC4K            | Source of Km resistance cartridge | Pharmacia |
| pQE30            | Vector for expression of His-tagged proteins, Cb<sup>R</sup> | Qiagen |
| pyncC<sub>HIS</sub> | pQE30::yncC expresses a His<sub>6</sub>-YncC protein, Cb<sup>R</sup> | 24 |
| pmcb<sub>R</sub><sub>HIS</sub> | pCA24N P<sub>TE-lac</sub>::yncC, C<sup>R</sup>, lac<sup>R</sup> | 25 |
| pCABg            | pmcb<sub>R</sub>HIS deleted from the BglII fragment carrying yncC | 21 |
| pJCD01           | Cloning vector for promoter fragments upstream of the rmb gene | 21 |
| pJCD<sub>yciG</sub>C | Cloning vector for promoter fragments upstream of the rmb gene | 21 |

### Table II

| Primer | Sequence |
|--------|----------|
| Ync<sup>H</sup>-E1 | GGAATTCCTGCGATTTTCTCCCGTTGCGAATAGTGCTGGCCCTGAGTCGCTTC |
| Ync<sup>H</sup>-E2 | GGAATTCCTGCGATTTTCTCCCGTTGCGAATAGTGCTGGCCCTGAGTCGCTTC |
| Ync<sup>H</sup>-H3 | GCCAAGAGATGAGGAGATTCTCGTCTGCCAGAAAGGTC |
| Ync<sup>H</sup>-H5 | AGTCAAGGCTCATGAAAGGATCTCGTCTGCCAGAAAGGTC |
| J7 (pJCD<sub>01</sub>) | GCCAAGAGATGAGGAGATTCTCGTCTGCCAGAAAGGTC |
| J7 (pJCD<sub>01</sub>) | GCCAAGAGATGAGGAGATTCTCGTCTGCCAGAAAGGTC |
| M47 (yciG<sub>STM</sub> up) | GCCAAGAGATGAGGAGATTCTCGTCTGCCAGAAAGGTC |
| M48bis (yciG<sub>STM</sub> down) | GCCAAGAGATGAGGAGATTCTCGTCTGCCAGAAAGGTC |
| M57bis (yciG<sub>STM</sub> middle) | GCCAAGAGATGAGGAGATTCTCGTCTGCCAGAAAGGTC |
| M91 (yciG<sub>COLI</sub> up) | GCCAAGAGATGAGGAGATTCTCGTCTGCCAGAAAGGTC |
| M92 (yciG<sub>COLI</sub> down) | GCCAAGAGATGAGGAGATTCTCGTCTGCCAGAAAGGTC |

The following primers were used for PCR in this study:

- Ync<sup>H</sup>-P1
- Ync<sup>H</sup>-P2
- YciG-P1
- Ync<sup>H</sup>-E1
- KatN-P1
- KatN-P2
- Hns-P1
- Hns-P2
- Ync<sup>H</sup>-H3
- Ync<sup>H</sup>-H5
- E7 (pJCD<sub>01</sub>)
- J7 (pJCD<sub>01</sub>)
- M47 (yciG<sub>STM</sub> up)
- M48bis (yciG<sub>STM</sub> down)
- M57bis (yciG<sub>STM</sub> middle)
- M91 (yciG<sub>COLI</sub> up)
- M92 (yciG<sub>COLI</sub> down)

A 50% acetonitrile-0.5% trifluoroacetic acid was applied twice on each spot and the spots were air dried. Molecules retained on the surfaces were visualized by reading the spots of each array in a SELDI-TOF-MS reader (PSC4000; Ciphergen Biosystems, Copenhagen, Denmark). Spectra were generated by seven shots on 36 pixels at laser energy varying between 2600 and 4500 nJ and an accelerating voltage of 25 kV in positive mode with automatic data collection software 3.0 program. External mass calibration was performed on one spot of each array by using ubiquitin (8564.8 Da), cytochrome C (12,230.9 Da), β-lactoglobulin A (18,363.3 Da), horseradish peroxidase (43,240.0 Da), Conalbumin (77,490.0 Da), and IgG bovine (147,300.0 Da).
Wizard operates in three passes across the spectra. The first pass performs peak detection at high signal-to-noise (s/n) ratio to pick out well-defined peaks as starting points for forming clusters. A second pass selects lower s/n ratio peaks, within a mass window defined around the first pass peaks. The algorithm completes the clusters in a third pass by creating artificial peaks where none were detected in the first two passes, at the exact center of clusters. In this analysis, unless otherwise specified, the first pass was performed with an s/n threshold of five, and the second pass with an s/n threshold of two, in a 0.5% width mass window. Clusters were assembled between 5000 and 40,000 Da. The cluster lists contained normalized peak intensity values for each sample within a group and p values were calculated between the medians of the peak intensities to detect significant differences in abundance for particular proteins.

Proteome Fractionation on Q Ceramic HYPERF F—A volume of 80 μl of Q Ceramic HYPERF F beads (BioSepara-Pall Corporation) were equilibrated three times in buffer T50, centrifuged, and resuspended in 10 ml of T50 containing 0.1% Triton. Bacterial cytosol extracts (8 mg in 4 ml) were incubated with the beads for 2 h at 4 °C on a rotative shaker and centrifuged. Beads were washed twice in T50 containing 0.1% Triton, and then twice in T50 and finally once in Tris-HCl 5 mm pH 9.0. Proteins captured on the beads were eluted successively in 100 μl HEPES 50 mm pH 8.0, 100 μl phosphate buffer 50 mm pH 7.0, 100 μl MES 50 mm pH 6.0, 100 μl sodium acetate 50 mm pH 5.0, 100 μl sodium acetate 50 mm pH 4.0, 100 μl sodium acetate 50 mm pH 3.4, and finally 100 μl sodium acetate 50 mm NaCl 1 m pH 3.4. Proteins in the eluted fractions (25 μl) were separated by SDS-PAGE on a 12.5% acrylamide gel. The gel was stained with Coomassie blue.

Identification of the YciF and YciE Proteins by Mass Spectrometry—Mass spectrometry analyses have been conducted at the PF3 Proteomic platform ( Abdelkader Namane, Institut Pasteur, France ).

Sample Preparation—One-dimensional gel bands were excised from gels and collected in 96-well plate. Destaining, reduction, alkylation, tryptic digestion of the proteins followed by peptide extraction were carried out with the Progest Investigator (Genomic Solutions, Ann Arbor, MI). Following the desalting step (C18-μZipTip, Millipore) peptides were eluted directly using the ProMS Investigator, (Genomic Solutions) onto a 96-well stainless steel matrix-assisted laser desorption ionization target plate (Applied Biosystems/MDS SCIEX, Framingham, MA) with 0.5 μl of CHCA matrix (5 mg/ml in 70% acetonitrile/30% H2O/0.1% trifluoroacetic acid).

MS and MS/MS Analysis—Raw data for protein identification were obtained on the 4800 Proteome Analyzers (Applied Biosystems) and analyzed by GPS Explorer 2.0 software (Applied Biosystems/MDS SCIEX). For positive-ion reflector mode spectra 3000 laser shots were averaged. For MS calibration, autolysis peaks of trypsin ([M+H]+ = 842.5100 and 2211.1046) were used as internal calibrates. Monoisotopic peak masses were automatically determined within the mass range 800–4000 Da with a signal to noise ratio minimum set to 20. Up to 12 of the most intense ion signals were selected as precursors for MS/MS acquisition enabling common trypsin autolysis peaks and matrix ion signals. In MS/MS positive ion mode, 4000 spectra were averaged. Collision energy was 2 kV, collision gas was air and default matrix ion signals. In MS/MS positive ion mode, 4000 spectra were averaged. For MS calibration, autolysis peaks of trypsin ([M+H]+ = 842.5100 and 2211.1046) were used as internal calibrates. 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mipetion of the same fragment treated for the A+G sequencing reaction (35). The E. coli H-N protein was a gift from Sylvie Rimsky and its concentration is expressed in monomers. In the competitive binding assays with H-NS and His₆-YncC, the proteins were first mixed and incubated with promoter fragments at 30 °C for 20 min in buffer C × 0.5 before DNase I attack.

In Vitro Transcription—The DNA fragments were prepared from pJCD01 derivatives containing the promoter fragments cloned upstream of the rmb1 terminator using primers E7 and J7 (25). A shorter S. Typhimurium yciG fragment (–61 to +48) that does not harbor the YncC binding site was generated using pJCDkatN and primers M57bis and J7 (21). The katE promoter fragment was described in Robbe-Saule et al. (21). E₉₉ RNA polymerase was reconstituted from core RNA polymerase either wild-type (Epicenter Biotechnologies, Madison, WI) or harboring α subunits truncated in the C-terminal domains (36), and His₆-α prepared from S. Typhimurium (37). Different amounts of YncC were incubated for 20 min at 37 °C with promoter fragments (at a final concentration of 10 nm) in buffer C before E₉₉ RNA polymerase addition (E: 15 nm, α₆: 60 nm) and incubation was prolonged for 20 min before addition of the heparin/XTP mixture (21). H-NS was first incubated with DNA templates (20 nm) for 20 min at 30 °C in 5 μl buffer A. A 5-μl aliquot of a mixture containing RNA polymerase with or without YncC (E: 60 nm, α₆: 240 nm, YncC: 550 nm) was then added. Incubation was prolonged for 10 min before adding 5 μl of a heparin/XTP mixture (450 μM ATP, CTP, and GTP and 45 μM [α-³²P]-UTP). The reaction was stopped following 10 min by adding 15 μl of formamide containing 10 μM EDTA, 1.6% SDS, and 0.02% xylene cyanol blue. An aliquot was loaded on a 7% polyacrylamide sequencing gel.

RESULTS

Putative YncC targets revealed by Proteinchip SELDI-TOF analyses—In a search for genes regulated by α₆ in S. Typhimurium, we isolated a transposon insertion in the yncC gene (7, ATCC-F12) (Table I). SELDI-TOF ProteinChip technology was used to capture and analyze proteins from clear lysates of the wild-type strain ATCC14028 and its mutant derivative ATCC-F12 grown to stationary phase in LB at 37 °C. When spectra from ATCC14028 and ATCC-F12 were compared, three peaks were reproducibly detected with a higher intensity in ATCC14028 than in ATCC-F12 (peaks 1–3, data not shown). Mutant ATCCyncC, which contains a deletion of the yncC gene, was subsequently constructed (Table I) and compared with ATCC14028. The three peaks, 1, 2, and 3 of molecular sizes 18,641, 18,964, and 31,935 Da, respectively, in Fig. 1A were detected at higher intensity levels in ATCC14028 than in ATCCyncC (p value 0.004, 6 samples per strain as described under “Experimental Procedures”). Interestingly, these peaks were not detected at significant levels in the spectra of the ΔrpoS mutant of ATCC14028 (ATCCrpoS, Fig. 1A). Therefore, these peaks might correspond to proteins encoded by genes regulated by α₆ and YncC.

Identification of YncC Targets—Because peak 3 was detected in the wild-type extract with a low intensity, compared with the other two peaks (Fig. 1A), we first focused on the identification of peaks 1 and 2. To identify these proteins, a partial purification scheme was devised (Experimental Procedures) involving anion exchange chromatography of clear lysates of the wild-type strain and the rpoS and yncC mutants followed by separation on the basis of pl. In the pH 5 fraction, two bands corresponding to proteins of 20 and 17 kDa were detected at a higher intensity in the extract of the wild-type strain than in the yncC and rpoS mutant extracts (Fig. 1B, lanes 3, 5, and 6). The two bands (shown by stars on Fig. 1B) were cut from the gel for subsequent trypsin digestion and identification by mass spectrometry (Table III) (Experimental Procedures). The proteins were identified as the YciF and YciE proteins of Salmonella (Fig. 1B).

The YciF and YciE proteins are encoded by an operon, yciGFEkatN (12) (Fig. 1C). Their calculated molecular sizes (Fig. 1C) correspond to those predicted from SELDI-TOF. The elution of these proteins from the anion exchange resin at pH 5 (Fig. 1B) is consistent with the calculated pl of YciF (5.24) and YciE (5.14). In addition, the 20 and 17 kDa proteins were not detected in ATCC-F1, which has a polar transposon insertion in yciF (Fig. 1B, lane 4).

The last gene in the operon, katN, encodes a protein of 31,848 Da that might correspond to peak 3 detected by SELDI-TOF (Fig. 1A). To check that peaks 1, 2, and 3 are encoded by the yciGFEkatN locus, the proteome of strain ATCCΔyci, in which the entire yciGFEkatN operon is missing (Table I), was compared with that of the wild-type strain. The spectra of the two strains were similar, except that peaks 1, 2, and 3 were not detected in ATCCΔyci (Fig. 1A), indicating that these peaks corresponded to YciF, YciE, and KatN. Peak 3, corresponding to KatN, has a molecular size (31,935 Da) that is slightly higher than that expected (31,848 Da). This difference might result from the presence of manganese in KatN (12) or from unknown posttranslational modification(s).

The relative abundance of the three proteins (YciF> YciE> KatN, Fig. 1A), is consistent with the position of the genes in the yciGFEkatN operon and mRNA levels (12). These proteins were not detected at significant levels in the ΔrpoS mutant, in agreement with our previous finding that expression of the operon is highly dependent on α₆ (12, 21). The YciG protein (6 KDa), encoded by the operon, was not detected, likely because conditions used for SELDI-TOF analyses were optimized for accurate detection of proteins > 10 kDa and because the calculated pl of YciG (9.99) is too high for it to bind to the anion exchange array.

YncC is Required for Maximal Transcription of the yciGFEkatN Operon in Salmonella—Results described earlier indicate that YciF, YciE, and KatN production is positively regulated by YncC. To determine whether this regulation operates at the transcriptional level, kinetics of expression of a katN-lacZ gene fusion in the wild-type strain and the ΔyncC mutant were compared. Expression of the fusion was delayed during early growth stages and was reduced by the ΔyncC mutation (Fig. 2A). The yncC gene in pACKyncC complemented the ΔyncC mutation for katN-lacZ expression, confirming that yncC activates katN transcription (Fig. 2B). In the wild-type strain, expression of katN-lacZ was induced earlier during growth and its expression level increased when yncC was ex-
pressed in trans from pACKyncC, suggesting that YncC might be a limiting factor for katN-lacZ expression under these conditions (Fig. 2B). As expected (12), katN-lacZ was expressed at very low levels in the absence of /H9268S (Fig. 2F, lanes 1 and 2). Neither the ΔyncC mutation nor pACKyncC had any effect on the expression of lacZ fused to katE, a catalase encoding gene also regulated by σ^5 and used as a control (data not shown). Altogether, these results suggest that YncC exerts a positive effect on yciGFEkatN operon transcription.

YncC Binds Upstream of the yciGFekatN Promoter—Plasmid pyncC_HIS encodes a recombinant YncC protein contain-

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**Fig. 1. Identification of protein targets for YncC regulation in Salmonella.** A, Selective capture of proteins from cell lysates of S. Typhimurium wild-type and mutant strains onto Q10 ProteinChip Array. Clear lysates from ATCC14028 (WT) and its mutant derivatives ATCCyncC, ATCCrpoS and ATCCicy were applied to the surface of a Q10 ProteinChip as described in the Experimental Procedures section. The captured proteins were detected using surface enhanced laser desorption/ionization (SELDI) time-of-flight mass spectrometry. The normalized mass (m/z) for each peak (in Da) is demonstrated on the x-axis, whereas intensity (µA) is plotted on the y-axis. Extracts were examined several times at different laser energies, and energies of 4500 nJ and 3200 nJ were found to be optimal for detection of peaks 1, 2, and peak 3, respectively. To pick out peak 3, the first pass peak detection was performed with a s/n threshold of two instead of five (Experimental Procedures). The relevant portion of the spectra from a representative experiment is shown. Similar results were obtained in two other experiments. The arrows indicate proteins expressed at different levels in the wild-type and the mutant strains. B, Partial purification of proteins of interest for identification by mass spectrometry. The pH 5 fraction (25 μl) of the Q Ceramic HYPERD F exchange resin from lysates of 2: ATCC katN-lacZ, 3: ATCC14028; 4: ATCC-F1; 5: ATCC-F12; and 6: ATCCrpoS were loaded on the gel. 1: molecular weight markers (in kDa). The stars indicate the protein bands that were excised from the gel and identified as the YciF and YciE proteins by mass spectrometry. C, Schematic representation of the yciGF katN loci in Salmonella (STM) and E. coli K-12 (ECO). The molecular sizes of the gene products are indicated in Daltons (Da). D, The yciG promoters in Salmonella and E. coli K-12. The relevant portion of the sequence in Salmonella ATCC14028 (STM) and E. coli MG1655 (ECO) is shown.

**TABLE III**

| Band | Protein name | Accession number | Matched/ searched | Sequence coverage % | Mascot Protein Score | Protein Confidence Index % | Sequence confirmed by CID | Mascot Ion Score |
|------|--------------|------------------|-------------------|---------------------|----------------------|---------------------------|--------------------------|-----------------|
| 1    | YciF         | gi16760152       | 5/21              | 37                  | 247                  | 100                       | 83                       |                 |
| 2    | YciE         | gi16760151       | 7/17              | 51                  | 191                  | 100                       | 128                      | 73              |

^a Bands are shown in Fig. 1B.

^b Genbank accession numbers for protein sequences 100% identical to that in Salmonella strain ATCC14028 (predicted from the nucleotide sequence of the genome, 29).

^c Detailed data are shown in Tables 4 and 5 (supplementary data).
ing six histidine residues at its N terminus (His6-YncC), under the control of the IPTG-inducible promoter of pQE30. pync-C_{HIS}, but not the pQE30 vector, complemented the \( \Delta \)yncC strain for \( \text{katN-lacZ} \) expression and increased \( \text{katN-lacZ} \) expression in the wild-type strain (Fig. 2F, lanes 9 and 10 and data not shown), indicating that His6-YncC is active. The recombinant protein was over-produced in \( E.\ coli \), purified and used for DNase I footprinting experiments on both strands of the \( S.\ typhimurium \) yciG promoter region (yciG_{STM}) (Fig. 3). His6-YncC protected a 24 bp sequence centered on the –100 region (Fig. 3B) relative to the transcription start site (14). The YncC binding site is AT-rich and contains the inverted repeat AATATAT. As expected, a footprint was not detected in the \( \text{katE} \) upstream promoter region (data not shown).

YncC also Binds to the \( E.\ coli \) K-12 yciGFE Promoter Region—The ortholog of yncC in \( E.\ coli \) K-12 (named mcbR),
Regulation of yciGFEkatN in Salmonella and E. coli

Regulates colanic acid production by repressing expression of the ybiM gene (13). Deletion of mcbR in E. coli MG1655 elicited mucoidy and decreased biofilm formation because of overproduction of colanic acid (13). The yncC mutants of Salmonella, ATCC-F12 and ATCCyncC, were not mucoid (data not shown), consistent with the absence of ybiM from S. Typhimurium genome (http://www.ncbi.nlm.nih.gov/).

In both Salmonella and E. coli K-12, yncC/mcbR is located between the yncB and yncD genes that encode a putative oxidoreductase and a putative iron outer membrane transporter, respectively. However, the intergenic regions between yncB and either yncC in Salmonella or mcbR in E. coli K-12 MG1655, which likely contain the yncC/mcbR promoter, differ in length and sequence, suggesting that yncC and mcbR might be differentially regulated. In addition, the amino acid sequences of YncC and McbR diverge in the C-terminal domain (46% identity over amino acids 78 to 221), compared with the N-terminal domain (81% identity over amino acids 1 to 77), which contains the predicted DNA binding HTH domain (8, 9). The C terminus of regulators of the GntR/FadR family contains an effector-binding and/or oligomerisation domain that influences the DNA-binding properties of the regulator (8–10). Altogether, these findings suggested that YncC and McbR might have evolved to respond to different signals and/or perform different functions in E. coli K-12 and Salmonella.

The yciGFE locus is conserved in E. coli K-12, but katN is not (Fig. 1C). The –35 and –10 elements of the yciGFEkatN promoter in Salmonella (yciGSTM, 12) are conserved in E. coli K-12 (Fig. 1D). However, the DNA region upstream of the –35 element, including the YncC binding site identified upstream of yciGSTM, diverge in the two species. Nevertheless, the His6-YncC protein could bind to the yciGECO promoter region (yciGECO) (Fig. 3). The footprint containing two repeats of the AATATAAT motif extended over 42 bp with two hypersensitive bands located on the nontemplate strand at the center of the protected region.

YncC belongs to the GntR subfamily of FadR (pfam007729), which binds as a dimer to its operator site via its winged helix domains (10). In the x-ray structure of the FadR-operator complex, the two recognition helices of each monomer project into a central major groove and the two β ribbons of the wings into the flanking minor grooves resulting in specific DNA-protein interactions over 11 bp. Based on these data, YncC likely binds DNA as a dimer and we predict that at least two tandem YncC operator sites are present upstream of the yciGECO promoter. The protection footprint of about 20 bp at yciGSTM suggests the presence of a single YncC binding site. Interestingly the YncC binding sites are located closer to the transcription start site at yciGECO than at yciGSTM (Fig. 3B).

The E. coli K-12 His6-McbR protein and the Salmonella His6-YncC protein showed similar protection footprint patterns at the yciGSTM and yciGECO promoters (data not shown), a finding consistent with the high sequence conservation in the DNA binding domains of these proteins.

The yciGFE Locus is Poorly Expressed in E. coli K-12—To determine the functional relevance of YncC/McbR binding to the yciGFE promoter region in E. coli K-12, the ability of E. coli MG1655 wild-type strain and mutant derivatives to produce the YciF and YciE proteins was assessed by SELDI-TOF technology. However, the spectra obtained for the wild-type MG1655, which likely contain the yncC/mcbR promoter, differed in length and sequence, suggesting that yncC and mcbR might be differentially regulated. In addition, the amino acid sequences of YncC and McbR diverge in the C-terminal domain (46% identity over amino acids 78 to 221), compared with the N-terminal domain (81% identity over amino acids 1 to 77), which contains the predicted DNA binding HTH domain (8, 9). The C terminus of regulators of the GntR/FadR family contains an effector-binding and/or oligomerisation domain that influences the DNA-binding properties of the regulator (8–10). Altogether, these findings suggested that YncC and McbR might have evolved to respond to different signals and/or perform different functions in E. coli K-12 and Salmonella.

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Fig. 4. Expression of the yciGFE locus in E. coli K-12 strains. A, SELDI-TOF-MS profiles of E. coli MG1655 and its mutant derivatives using the Q10 ProteinChip array. Clear lysates from MG1655 (WT) and its mutants MG655hns, MG655hnsyciE, and MG655hnsyciE were applied to the surface of a Q10 ProteinChip as described in the Experimental Procedures section. The captured proteins were detected using surface enhanced laser desorption/ionization (SELDI) time-of-flight mass spectrometry. Normalized mass (m/z) for each peak (in Daltons (Da)) is demonstrated on the x-axis, whereas intensity (μA) is plotted on the y-axis. A laser energy of 3200 nJ was used. The relevant portion of the spectra is shown. The arrows indicate the YciE and YciF proteins. B, Expression of the yciE-lacZ fusion in the E. coli strains indicated was determined in overnight LB cultures at 37 °C. Lanes 1 to 5: (1) MC4100 yciE-lacZ, (2) MC4100hns yciE-lacZ, (3) MC4100mcbR yciE-lacZ, (4) MC4100hnsmbcR yciE-lacZ, (5) MC4100hnsrpoS yciE-lacZ. Lanes 6 to 15: MC4100 yciE-lacZ harboring pCABg (6), pmbcR_{HIS} (7), pQE30 (12), and pyncC_{HIS} (13); MC4100hns yciE-lacZ harboring pCABg (8), pmbcR_{HIS} (9), pQE30 (14), and pyncC_{HIS} (15); and MC4100hnsmbcR yciE-lacZ harboring pCABg (10), and pmbcR_{HIS} (11).

Genes in E. coli K-12 further, a chromosomal yciE-lacZ transcriptional fusion was constructed in two E. coli K-12 Lac- strains, MC4100 and MC1061. The fusion was expressed at very low levels in both strains and in their ΔmcbR and ΔrpoS mutant derivatives (Fig. 4B, lane 1 and data not shown). The His_{6}-McbR and His_{6}-YncC proteins were able to induce yciE-lacZ expression, although the levels of expression remained low (Fig. 4B, lanes 6 and 7 and 12 and 13, respectively). yciE-lacZ expression was induced to higher levels by His_{6}-McbR than by His_{6}-YncC (Fig. 4B, lanes 7 and 13), likely because the former is more abundant (Fig. 5A). Like the His_{6}-YncC protein, the His_{6}-McbR protein activated expression of the katN-lacZ fusion in the ΔyncC and wild-type strains of Salmonella (Fig. 2C). Altogether, these results show that YncC and McbR are both able to induce expression of the yciGFE(katN) locus in Salmonella and E. coli K-12, and that this locus is expressed at drastically lower levels in E. coli K-12 than in Salmonella.

Gene Polymorphism at the trpA-yciGFE-ompW Locus in E. coli Strains—The low GC content of the yciF and yciE genes relative to the resident genome suggested that these genes were horizontally acquired in E. coli K-12 (38) and Salmonella (39, 40). Interestingly, in nearly half of the 23 complete sequenced genomes of E. coli strains (http://www.
ncbi.nlm.nih.gov/), the trpA-yciGFE-ompW locus (Fig. 1C) is a site of DNA rearrangements, including deletions and/or insertions of phage-related and/or virulence-associated genes. In addition, in pathogenic E. coli O157:H7, a second copy of the yciGFE locus, including the katN gene, is located on a cryptic prophage (CP-933X) elsewhere in the genome. The sequence identity between the prophage-borne gene products in E. coli O157:H7 and corresponding gene products in E. coli K-12 and Salmonella is high (more than 80%). However, the noncoding sequence upstream of the prophage-borne yciGFEkatN genes is different from those upstream of yciGECO and yciGSTM and does not contain the –10 and –35 promoter elements present in the two other loci (data not shown). The yciGFEkatN genes are absent from the closest E. coli relative, E. fergusonii (http://www.ncbi.nlm.nih.gov/). Altogether, these findings are consistent with horizontal acquisition of these genes in E. coli.

In contrast to the situation in E. coli, the yciGFEkatN locus belongs to the core genome in Salmonella (41), suggesting that Salmonella acquired these genes before the lineage divided into the two Salmonella species, S. enterica and S. bongori. The 479 pb sequence between trpA and yciG (Fig. 1C) is also conserved in the 16 complete sequenced genomes of S. enterica (http://www.ncbi.nlm.nih.gov/) and in S. bongori (http://www.sanger.ac.uk/Projects/Salmonella/). The sequence of the –10 and –35 elements is identical in all these Salmonella genomes. The sequence of the YncC binding region (in yciGSTM, Fig. 3B) is 100% identical in the genomes of S. enterica subsp. enterica and only one mismatch is found at the boundaries of this motif (Fig. 3B) in the two most ancestral groups (41), S. enterica subsp. arizonae (T/C at position –91) and S. bongori (G/A at position –110).

H-NS Silencing of yciGFEkatN is Relieved in Stationary Phase by αH in Salmonella—H-NS is an abundant histone-like protein that binds preferentially to AT-rich DNA and subsequently oligomerizes along the DNA resulting in the formation of extended nucleoprotein complexes that cause gene repression (14–16). Preferential binding of H-NS to sequences with higher AT-content than the resident genome allows H-NS to repress the expression of foreign DNA in a process known as “xenogeneic silencing” (15, 16). Selective silencing of foreign DNA with low GC content in Salmonella by H-NS has been reported using chromatin immunoprecipitation and microarray analyses (39, 40). Examination of these data revealed that H-NS binds the yciGFEkatN locus and represses its expression.

In agreement with these findings, expression of katN-lacZ in S. Typhimurium ATCC14028 (Fig. 2D) and SL1344 (data not shown) was increased by an hns mutation by more than 10-fold in exponential phase and by twofold in stationary phase. In the hns mutants, αH was still required for katN-lacZ expression (Fig. 2D and data not shown). The growth rate of Salmonella was highly affected by the hns mutation (Fig. 2D). H-NS is required for the normal proteolytic turnover of αH (42) and, thus, levels of αH in the exponential phase were higher in the hns strains than in the wild-type strains (Fig. 5D, lanes 5 and 7). As previously reported (39, 40), the high αH content in the hns strain likely impairs growth, because the growth defect of the hns mutant can be partially alleviated by deleting rpoS (Fig. 2D) or by replacing the wild-type rpoS allele by the rpoS_{L72} allele (Fig. 2E). The rpoS_{L72} allele contains a rare TTG start codon (instead of ATG). This mutation lowered the αH level in ATCCrpoSL72 and ATCCrpoSL72hns in exponential phase (Fig. 5D, lanes 5, 7, and 11, and lanes 7 and 13) and to a lesser extent in stationary phase (21) (Fig. 5C, lanes 5 and 11–14). In stationary phase, derepression of katN-lacZ expression by the hns mutation was stronger in ATCCrpoSL72 than in ATCC14028 (12- and twofold respectively, Figs. 2D, E). However, αH levels and katN-lacZ expression levels in stationary phase were not highly different in the two hns strains (twofold difference) (Figs. 2D, E and Fig. 5C lanes 13 and 14). These results suggested that the reduction in αH level, because of the rpoS_{L72} mutation, potentiated the magnitude of repression of katN-lacZ expression by H-NS.

αH is not Efficient to Counter H-NS Silencing of yciGFE in E. coli K-12—Two peaks of 18,590 and 18,961 Da, likely corresponding to the YciF and YciE proteins, were detected by SELDI-TOF ProteinChip analyses in the Δhns mutant of MG1655 but not in the ΔhnsΔyciF and ΔhnsΔyciE mutants respectively (Fig. 4A). Detection of the YciE and YciF proteins in the hns mutant but not in the wild-type strain (Fig. 4A) suggested that expression of these proteins is silenced by H-NS in E. coli K-12, in agreement with a previous finding (43). Expression of the yciE-lacZ fusion was consistently derepressed in the hns mutant of MC4100 (Fig. 4B, lanes 1 and 2). Expression of the yciE-lacZ fusion in the hns strain was strongly affected by the rpoS mutation (Fig. 4B, lanes 2 and 5). These results indicated that αH induces expression of yciGFE in E. coli K-12 only when H-NS-mediated repression was relieved. Repression of yciGFEkatN by H-NS is inversely correlated with the αH levels (Figs. 2D, E and Figs. 5C, D), and, thus, one possible explanation for these results is that E. coli produces less αH than Salmonella. This hypothesis was ruled out because similar levels of αH were detected in MG1655, MC4100, MC1061, and ATCC14028 (Figs. 5B, C, D). In addition, similar levels of αH were detected in the hns derivatives of these strains (Fig. 5B and data not shown).

YncC Directly Activates E_{αH}-dependent In Vivo Transcription at yciGSTM—Significant expression of the yciGFEkatN operon was not detected in the ΔrpoS mutant of Salmonella (12) (Fig. 2F, lane 2), and katN-lacZ expression was very low in the rpoShns strain of Salmonella (Figs. 2D, E). These results suggested that yciGSTM is not efficiently transcribed in the absence of αH. In agreement with this conclusion, a transcript initiating at yciGSTM was detected in vitro with the αH-holoenzyme (E_{αH}) but not with αH_70-holoenzyme (E_{αH_70}) (Fig. 6B).

In plasmids pACKyncC and pyncCHIS, yncC lacks its own promoter and is transcribed from the promoters in the vectors.
These results suggested that transcription activation by YncC requires the αCTD domain.

YncC activates transcription at pyciGECO by counteracting H-NS-mediated silencing—The core promoter regions of pyciGECO and pyciGSTM are very similar (Fig. 1D) and, as expected, transcription initiation occurred at similar sites in vitro (Fig. 7). Like pyciGSTM, pyciGECO was selectively transcribed by Eσ^5 (data not shown). However, in contrast to pyciGSTM, pyciGECO was not activated by YncC (Fig. 7, lanes 1 and 2 and data not shown). Addition of H-NS strongly decreased transcription by Eσ^5 at pyciGECO (5- to 100-fold) (Fig. 7, lanes 1, 3, 5, and 7), as expected from in vivo data (Fig. 4). YncC decreased the magnitude of H-NS-mediated repression (Fig. 7, lanes 3–8). The katE promoter, used as a control, was hardly repressed by H-NS and was insensitive to YncC (Fig. 7, lanes 17–24), in agreement with in vivo data (not shown).

These results suggested that activation of yciE-lacZ expression in E. coli by His^S-McbR and His^S-YncC (Fig. 4B) was because of the ability of these proteins to counter H-NS-mediated silencing at pyciGECO. Consistent with this hypothesis, YncC/McbR did not have a marked effect on yciGFE expression in E. coli K-12 in the absence of H-NS (Fig. 4B, lanes 2 and 4, lanes 6–11 and lanes 12–15).

On binding to high affinity “nucleation” sites, H-NS spreads along the DNA to lower affinity sites to occupy the promoter region, allowing the formation of higher order structures. An H-NS binding region contains several sites with variable affinity, and the number and the organization of binding sites determines the formation of a repressive nucleoprotein complex and modulates H-NS repression of gene expression (14–16). DNase I footprinting experiments showed that H-NS binds to the pyciGECO promoter region (Fig. 8, lanes 2–5). The binding pattern was marked by a series of protected and hypersensitive bands, indicating that H-NS changes the local topology of the promoter region. The polymerization of H-NS molecules along the promoter sequence would be expected to repress yciGFE expression by promoter occlusion or by antagonizing open complex formation. According to the 10-bp consensus binding sequence reported for H-NS nucleation sites (44), two predicted H-NS binding sites of high affinity are located in the upstream promoter region, precisely in the motifs recognized by YncC/McbR. The first site, located in the upstream YncC box AATATATC (Fig. 3B), matches the consensus over 8 bp, whereas the other, AATATATttt in the downstream YncC box (Fig. 3B), has 6 bp matches and, therefore, is expected to have lower affinity (note that the bases matching the H-NS consensus site are indicated in capital letters).

Many DNA-binding proteins can counter H-NS-mediated silencing, usually through competition for H-NS binding (15, 45). DNase I footprinting experiments with both H-NS and YncC showed that they compete for DNA binding (Fig. 8,

Regulation of yciGFEkatN in Salmonella and E. coli
Regulation of yciGFEkatN in Salmonella and E. coli

![Graph](image.png)

**Fig. 7. Differential effects of YncC on H-NS-mediated repression.** Single round run-off transcripts using DNA fragments containing the promoter regions of yciGSTM (~184 to +48), yciGECO (~227 to +66) and katE (~106 to +66). The templates were first incubated with or without H-NS for 20 min: lanes 1, 2, 9, 10, 17, and 18; no H-NS; lanes 3, 4, 11, 12, 19, and 20, 150 nM H-NS; lanes 5, 6, 13, 14, 21, and 22, 250 nM H-NS; lanes 7, 8, 15, 16, 23, and 24, 350 nM H-NS. The α5-RNA polymerase (E: 30 nM; α5’: 120 nM) was added alone or in combination with YncC (250 nM) and incubation was prolonged for 10 min before addition of the heparin/XTP mixture. The histograms and number below each lane show the quantification of the transcripts and the effects of H-NS and YncC.

lanes 8, 9, and 10). YncC prevented H-NS binding only at high concentrations (Fig. 8, compare lanes 10 and 11). These results suggested that, when present in high amounts in *E. coli*, YncC/McbR counters H-NS mediated-repression at pyciG~ECO~ by modulating H-NS binding and, ultimately, by counteracting the negative effects of H-NS.

**Interplay Between H-NS and YncC at the yciGSTM Promoter—In vitro repression by H-NS was less marked at pyciGSTM than at pyciG~ECO~ (Fig. 7), a result consistent with in vivo data (Figs. 2F and 4B). The magnitude of H-NS repression was not affected by YncC (Fig. 7), and the magnitude of YncC activation was not affected by H-NS (Fig. 7). H-NS was able to bind to pyciGSTM, protecting multiple sites along the DNA leading to the formation of a repression complex (Fig. 8, lanes 12 to 16). As for the pyciG~ECO~, two predicted H-NS binding sites (agaATATTT centered at –98.5 and AtaTTATCTc centered at –93.5) in pyciGSTM overlap the single YncC binding site (Fig. 3B). In the competitive footprinting assay between the two proteins, YncC occupied the pyciGSTM site at a lower concentration than at pyciG~ECO~, but this did not appear to prevent H-NS binding at other sites along the promoter fragment (Fig. 8, lanes 20–22). Indeed, some bands around –88, which are not protected by YncC or H-NS alone, are protected in the combined footprint, supporting the notion that H-NS and YncC did not compete for binding but rather bound simultaneously to pyciGSTM fragment (Fig. 8, compare lanes 18 and 22). These results are in agreement with the in vitro transcription data (Fig. 7), and suggest that YncC activation and H-NS repression occur independently at pyciGSTM.

The effect of the ΔyncC mutation on katN-lacZ expression in *Salmonella* was attenuated in the absence of H-NS (Figs. 2A, D, E). One possible explanation for this result is that the greater abundance of α5 in the *hns* strain compared with the wild-type strain (Fig. 5D) reduced the need for YncC in the absence of H-NS. Indeed, the impact of YncC on katN-lacZ expression was greatest at the entry to stationary phase (Figs. 2A, B), when α5 begins to accumulate in the cells (21), suggesting that the impact of YncC might be greatest at low α5 concentrations. Consistent with this hypothesis, katN-lacZ transcription activation by Hls~S~YncC was higher in the presence of the rpoS~L72~ allele (ninefold) (Fig. 2F lanes 5 and 6) than in the presence of the wild-type rpoS allele (3.5-fold) (Fig. 2F lanes 9 and 10).

**DISCUSSION**

In the present study, a proteomic method using ProteinChip arrays coupled with surface-enhanced laser desorption/ionisation time of flight mass spectrometry (SELDI-TOF-MS) was used for comparative proteomic profiling of cell extracts from *Salmonella* strains. These experiments revealed three proteins, subsequently identified as the yciF, yciE, and katN gene products that were produced in lower amounts in the yncC mutant than in the wild-type strain. Gene fusion analyses and in vitro transcription and DNase I footprinting experiments demonstrated that YncC controls production of these proteins at the transcriptional level and acts in concert with α5. The α5 also controls expression of yncC (7), and thus, yciG-FEkatN is regulated by a α5-dependent feed-forward regulatory loop. This dual role of α5 in the control of the operon, and the inverse correlation, observed in this study, between the α5 level and the magnitude of H-NS repression, might account for the strong sensitivity of yciGFEkatN expression to α5 levels and to activation by Crl (21), the α5-chaperone that increases α5 activity.

Like most α5-dependent promoters, pyciGSTM had a moderate activity in vitro, which was slightly stimulated by binding of YncC to pyciGSTM (Figs. 3, 6, and 8), and required αCTD of
RNAP (Fig. 6). The results suggested that YncC might act as a class I activator, making direct interactions with CTD, thereby recruiting the rest of RNA polymerase (46). The location of activator binding in promoters subject to class I activation is variable, because of the flexibility of the linker between the N- and C- terminal domains of the subunit, but is usually near positions –61, –71, –81 or –91. The position of the YncC binding site, centered at –101 with respect to the transcription start site of yciG, is 10 bp upstream of the most distant transcription activators at simple 70-dependent promoters. However the intrinsic DNA curvature found in many S-regulated promoters (including pyciGSTM, data not shown) might facilitate protein-protein contacts between YncC and the CTD of the distal subunit of RNA polymerase, which is used preferentially by E. coli for activation (46–48).

H-NS and YncC can bind DNA simultaneously to regulate in vitro transcription at pyciGSTM (Figs. 7 and 8). In vivo however, the magnitude of YncC activation was reduced in the absence of H-NS (Figs. 2D, E). This might result from the high levels in the hns strains, or from the involvement of an additional molecule that regulates yciGFEkatN expression. Alternatively, H-NS not only binds to the promoter region of yciGFEkatN (Fig. 8) but also to coding regions (39, 40 and our unpublished results) and might form DNA bridges that contribute to transcription repression (14–16). Significant binding of YncC to the coding sequences tested so far was not observed (data not shown). YncC might help relieve H-NS silencing indirectly, by increasing the transcription initiation rate at pyciGSTM and, thus, the transcription elongation rate across the H-NS binding region, in line with the situation reported at the bgl promoter (49). Further experiments will evaluate the effect of downstream sequences in H-NS silencing of pyciGSTM.

YncC/McbR and H-NS are also able to bind to the promoter region of the E. coli K-12 yciGFE genes. However, the sequence, the length, and the position of the McbR/YncC and H-NS binding regions, relative to the yciGFE(katN) promoter, are different in E. coli K-12 and Salmonella, resulting in differential mechanisms of regulation of these genes by YncC/McbR and H-NS. It is remarkable that in E. coli K-12, regulation of yciGFE by YncC and by H-NS are intimately linked, whereas in Salmonella, YncC directly activates transcription, and thus, activation by YncC is, at least partly, disconnected.

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**Fig. 8.** DNase I footprint analysis of pyciGEco and pyciGSTM promoters with H-NS, YncC and both proteins. The 5'-radiolabeled promoter fragment (template strand) was incubated with H-NS, YncC or a preformed mixture of both proteins for 20 min before DNase I attack, lanes 1, 12, and 19: no protein; lanes 2 and 13: 37.5 nM H-NS; lanes 3 and 14: 75 nM H-NS; lanes 4 and 15: 150 nM H-NS; lanes 5, 11, and 16: 250 nM H-NS; lanes 6 and 17: 250 nM YncC; lanes 7 and 18: 1 µM YncC; lanes 8 and 20: 250 nM H-NS with 250 nM YncC; lanes 9 and 21: 250 nM H-NS with 500 nM YncC; lanes 10 and 22: 250 nM H-NS with 1 µM YncC. M is a Maxam-Gilbert A+G track and the numbering on the left is relative to the transcription start site. Open or black triangles (right side) indicate bands with decreased or enhanced intensity, respectively, in the presence of H-NS. The footprint of YncC is represented to the right by a solid black bar. The location of the bands that are only protected in the presence of both YncC and H-NS is marked with an open arrow.

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Regulation of yciGFEkatN in Salmonella and E. coli
from the H-NS network. One exciting possibility is that Salmonella has evolved the yciGFEkatN cis-regulatory sequences to integrate this locus into the RpoS network while maintaining its connection to the H-NS network, ultimately resulting in a more versatile but tightly controlled expression of this locus. The feed-forward regulatory loop mediated by YncC might allow signal input at levels downstream of σ52 expression, through modulation of YncC activity or expression. Our data show that YncC production and/or activity is a limiting factor for yciGFEkatN expression and that the impact of YncC is major at low σ52 concentrations. One hypothesis is that YncC induces yciGFEkatN expression under a specific environmental condition in the exponential phase of growth where σ52 level is low. Our future experiments will assess environmental signals that might modulate YncC activation of yciGFEkatN. These experiments might reveal putative cofactors that bind to the C-terminal domain of YncC and modulate its DNA binding activity.

The evolution of promoter architecture in closely related bacterial species might have important consequences for bacterial adaptation (50–52). The physiological role of the yciGFEkatN locus is unknown, and the significance, in the fitness of E. coli and Salmonella, of the differential regulation of these genes requires further investigation. Structural comparisons suggest a role for YciF in iron storage and/or protection against oxidative damage (53). KatN belongs to the family of manganese catalases but it does not play a major role in hydrogen peroxide resistance of Salmonella under standard growth conditions or in virulence in mice (12, 54). This is because of the functional redundancy of the five hydrogen peroxide scavengers (three catalases and two alkyl hydroperoxide reductases) that contribute to Salmonella virulence and oxidative stress resistance (54). Nevertheless, overproduction of KatN increased resistance of Salmonella to hydrogen peroxide (12) and enhanced its virulence in NF-κB pathway mutant Drosophila (55), suggesting that KatN might indeed contribute to Salmonella fitness. One could speculate on a correlation between gene polymorphism at the trpA yciGFE ompW locus in E. coli, silencing of yciGFE by H-NS, and the absence of katN. Investigation of the regulation and the role of the prophase-borne yciGFEkatN locus in the virulence and fitness of pathogenic E. coli O157:H7 strains might provide insight into the evolution and the function of this locus in closely related Enterobacteria.

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REFERENCES

1. Gruber, T.M., and Gross, C.A. (2003) Multiple sigma subunits and the partitioning of bacterial transcription space. Annu. Rev. Microbiol. 57, 441–466
2. Ishihama, A. (2000) Functional modulation of Escherichia coli RNA polymerase. Annu. Rev. Microbiol. 54, 499–518
3. Klauck, E., Typas, A., and Hengge, R. (2007) The sigmaS subunit of RNA polymerase as a signal integrator and network master regulator in the general stress response in Escherichia coli. Sci. Prog. 90, 103–127
4. Dong, T., and Schellhorn, H. E. (2010) Role of RpoS in virulence of pathogens. Infect. Immun. 78, 887–897
5. Bang, I.S., Frye, J.G., McClelland, M., Velayudhan, J., and Fang, F.C. (2005) Alternative sigma factor interactions in Salmonella: σE and σH promote antioxidant defences by enhancing σE levels. Mol. Microbiol. 56, 811–823
6. Weber, H., Polen, T., Heuveling, J., Wendisch, V. F., and Hengge, R. (2005) Genome-wide analysis of the general stress response network in Escherichia coli: σE-dependent genes, promoters, and σE factor selectivity. J. Bacteriol. 187, 1591–1603
7. Ibanez-Ruiz, M., Robbe-Saule, V., Hermant, D., Labrude, S., and Norel, F. (2000) Identification of RpoS (σE)-regulated genes in Salmonella enterica serovar Typhimurium. J. Bacteriol. 182, 5749–5756
8. Rigali, S., Derouaux, A., Giannotta, F., and Dusart, J. (2002) Subdivision of the helix-turn-helix GntR family of bacterial regulators in the FadR, HupC, MocR, and Yra subfamilies. J. Biol. Chem. 277, 12,507–12,515
9. Hoskisson, P.A., and Rigali, S. (2009) Chapter 1: Variation in form and function of the helix-turn-helix regulators of the GntR superfamily. Adv. Appl. Microbiol. 69, 1–22
10. Xu, Y., Heath, R. J., Li, Z., Rock, C. O., and White, S. W. (2001) The FadR-DNA complex. Transcriptional control of fatty acid metabolism in Escherichia coli. J. Biol. Chem. 276, 17373–17379
11. Poon, T. (2007) Opportunities and limitations of SELDI-TOF-MS in biomedical research: practical advice. Expert Rev. Proteomics. 4, 51–65
12. Robbe-Saule, V., Cynnaert, C., Ibanez-Ruiz, M., Hermant, D., and Norel, F. (2001) Identification of a non-α-haem catalase in Salmonella and its regulation by RpoS (σE). Mol. Microbiol. 39, 1533–1545
13. Zhang, X. S., Garcia-Contreras, R., and Wood, T. K. (2008) Escherichia coli transcription factor YncC (McbR) regulates colanic acid and biofilm formation by repressing expression of periplasmic protein YbiM (McBA). ISME J. 2, 615–631
14. Dorman, C. J. (2007) H-NS, the genome sentinel. Nat. Rev. Microbiol. 5, 157–161
15. Fang, F. C., and Rimsky, S. (2008) New insights into transcriptional regulation by H-NS. Curr. Opin. Microbiol. 11, 113–120
16. Dorman, C. J., and Kane, K. A. (2009) DNA bridging and anti-bridging: a role for bacterial nucleoid-associated proteins in regulating the expression of laterally acquired genes. FEMS Microbiol. Rev. 33, 587–592
17. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular cloning: A Laboratory Manual (2nd ed.). Cold Spring Harbor, N. Y.
18. Blattner, F. R., Plunkett, G., 3rd, Bloch, C. A., Perna, N. T., Burland, V., Riley, M., Collado-Vides, J., Glasner, J. D., Rode, C. K., Mayhew, G. F., Gregor, J., Davis, N. W., Kirkpatrick, H. A., Goeden, M. A., Rose, D. J., Mau, B., and Shao, Y. (1997) The complete genome sequence of Escherichia coli K-12. Science. 277, 1453–1462
19. Bertin, P., Terao, E., Lee, E. H., Lejeune, P., Colson, C., Danchin, A., and Collatz, E. (1994) The H-NS protein is involved in the biogenesis of flagella in Escherichia coli J. Bacteriol. 176, 5537–5540
20. Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datserko, K. A., Tomita, M., Wanner, B. L., and Mori, H. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. Mol. Syst. Biol. 2, 2006.0008
21. Robbe-Saule, V., Lopes, M. D., Kolb, A., and Norel, F. (2007) Physiological effects of Crl in Salmonella are modulated by sigmaS level and promoter specificity. J. Bacteriol. 189, 2976–2987
22. Robbe-Saule, V., Jaumouillle, V., Prévost, M. C., Guadagnini, S., Talhouarne, C., Mathout, H., Kolb, A., and Norel, F. (2006) Crl activates

Molecular & Cellular Proteomics 9.12
transcription initiation of RpoS-regulated genes involved in the multicellular behavior of Salmonella enterica serovar Typhimurium. J. Bacteriol. 188, 3983–3994

23. Chang, A. C., and Cohen, S. N. (1978) Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. 134, 1141–1156

24. Kitagawa, M., Ara, T., Arifuzzaman, M., Ioka-Nakamichi, T., Inamoto, E., Toyonaga, H., and Mori, H. (2005) Complete set of ORF clones of Escherichia coli ASKA library (a complete set of E. coli K-12 ORF archive); unique resources for biological research. DNA Res. 12, 291–299

25. Marschall, C., Labrousse, V., Kremer, M., Weichart, D., Kolb, A., and Hengge-Aronis, R. (1998) Molecular analysis of the regulation of csiD, a carbon starvation-inducible gene in Escherichia coli that is exclusively dependent on sigma S and requires activation by cAMP-CRP. J. Mol. Biol. 276, 339–353

26. Schmieg, H. (1972) Phase P22-mutants with increased or decreased transduction abilities. Mol. Gen. Genet. 119, 75–88

27. Sternberg, N. L., and Maurer, R. (1991) Bacteriophage-mediated generalized transduction in Escherichia coli and Salmonella typhimurium. Methods Enzymol. 204, 18–43

28. Silhavy, T. J., Berman, M. L., and Enqvist, L. W. (1984) Experiments with Gene Functions, Cold Spring Harbour, N. Y.

29. Jarvik, T., Smillie, C., Groisman, E. A., and Ochman, H. (2010) Short-term signatures of evolutionary change in the Salmonella enterica serovar Typhimurium 14028 genome. J. Bacteriol. 192, 560–567

30. Datsenko, K. A., and Wanner, B. L. (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. U.S.A. 97, 6640–6645

31. Ellermeier, C. D., Janakiraman, A., and Slauch, J. M. (2002) Construction of targeted single copy lac fusions using lambda Red and FLP-mediated site-specific recombination in bacteria. Gene. 290, 153–161

32. Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbour, N. Y.

33. Coyneault, C., Robbe-Saule, V., and Norel, F. (1996) Virulence and vaccine potential of Salmonella typhimurium mutants deficient in the expression of the RpoS (sigma S) regulon. Mol. Microbiol. 22, 149–160

34. Depardieu, F., Courvalin, P., and Kolb, A. (2005) Binding sites of VanRB and sigma70 RNA polymerase in the vanB vancomycin resistance operon of Enterococcus faecium BM4524. Mol. Microbiol. 57, 550–564

35. Maxam, A. M., and Gilbert, W. (1977) A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. 74, 560–564

36. Huo, Y. X., Tian, Z. X., Rappas, M., Wen, J., Chen, Y. C., You, C. H., Zhang, X., Buck, M., Wang, Y. P., and Kolb, A. (2006) Protein-induced DNA bending clarifies the architectural organization of the sigma54-dependent glnAp2 promoter. Mol. Microbiol. 59, 168–180

37. Monteil, V., Kolb, A., D’Alayer, J., Beguin, P., and Norel, F. (2010) Identification of conserved amino acid residues of the Salmonella sigmaS chaperone Crl involved in Crl-sigmaS interactions. J. Bacteriol. 192, 1075–1087

38. Lawrence, J. G., and Ochman, H. (1998) Molecular archaeology of the Escherichia coli genome. Proc. Natl. Acad. Sci. U.S.A. 95, 9413–9417

39. Lucchini, S., Rowley, G., Goldberg, M. D., Hurd, D., Harrison, M., and Hinton, J. C. (2006) H-NS mediates the silencing of laterally acquired genes in bacteria. PLoS Pathog. 2, e81

40. Navarre, W. W., Porwollik, S., Wang, Y., McClelland, M., Rosen, H., Libby, S. J., and Fang, F. C. (2006) Selective silencing of foreign DNA with low GC content by the H-NS protein in. Salmonella. Science. 313, 236–238

41. Chan, K., Baker, S., Kim, C. C., Detweiler, C. S., Dogan, G., and Falkow, S. (2003) Genomic comparison of Salmonella enterica serovars and Salmonella bongori by use of art s enterica serovar Typhimurium DNA microarray. J. Bacteriol. 185, 553–563

42. Zhou, Y., and Gottesman, S. (2006) Modes of regulation of RpoS by H-NS. J. Bacteriol. 188, 7022–7025

43. Yoshida, T., Ueguchi, C., Yamada, H., and Mizuno, T. (1993) Function of the Escherichia coli nucleoid protein, H-NS: molecular analysis of a subset of proteins whose expression is enhanced in a hns deletion mutant. Mol. Gen. Genet. 237, 113–122

44. Lang, B., Blot, N., Bouffartigues, E., Buckle, M., Geertz, M., Gualerzi, C. O., Mavathur, R., Mushkelishvili, G., Pon, C. L., Rimsky, S., Stella, S., Babu, M. M., and Travers, A. (2007) High-affinity DNA binding sites for H-NS provide a molecular basis for selective silencing within proteobacterial genomes. Nucleic Acids Res. 35, 6330–6337

45. Stoebel, D. M., Free, A., and Dorman, C. J. (2008) Anti-silencing: overcoming H-NS-mediated repression of transcription in Gram-negative enteric bacteria. Microbiology. 154, 2533–2545

46. Browning, D. F., and Busby, S. J. (2004) The regulation of bacterial transcription initiation. Nat. Rev. Microbiol. 2, 57–65

47. Espinosa-Urgel, M., and Tormo, A. (1993) Sigma S-dependent promoters in Escherichia coli are located in DNA regions with intrinsic curvature. Nucleic Acids Res. 21, 3667–3670

48. Tysia, A., Barembruch, C., Possling, A., and Hengge, R. (2007) Stationary phase reorganisation of the Escherichia coli transcription machinery by Crl protein, a fine-tuner of sigmaS activity and levels. EMBO J. 26, 1569–1578

49. Radde, N., Gebert, J., Faigle, U., Schrader, R., and Schnett, K. (2008) Modeling feedback loops in the H-NS-mediated regulation of the Escherichia coli bgl operon. J. Theor. Biol. 250, 298–306

50. Osborne, S. E., Watthers, D., Tomljenovic, A. M., Mulder, D. T., Sliphuadang, U., Duong, N., Lowden, M. J., Wickham, M. E., Waller, R. F., Kenney, L. J., and Coombes, B. K. (2009) Pathogenic adaptation of intracellular bacteria by rewiring a cis-regulatory input function. Proc. Natl. Acad. Sci. U.S.A. 106, 3982–3987

51. Perez, J. C., and Groisman, E. A. (2009) Transcription factor function and promoter architecture govern the evolution of bacterial regulons. Proc. Natl. Acad. Sci. U.S.A. 106, 4319–4324

52. Perez, J. C., and Groisman, E. A. (2009) Evolution of transcriptional regulatory circuits in bacteria. Cell. 138, 233–244

53. Hindupur, A., Liu, D., Zhao, Y., Bellamy, H. D., White, M. A., and Fox, R. O. (2006) The crystal structure of the E. coli stress protein YicF. Protein Sci. 15, 2605–2611

54. Hébrard, M., Viala, J. P., Méresse, S., Barras, F., and Aussel, L. (2009) Redundant hydrogen peroxide scavengers contribute to Salmonella virulence and oxidative stress resistance. J. Bacteriol. 191, 4605–4614

55. Ryu, J. H., Ha, E. M., Oh, C. T., Seol, J. H., Brey, P. T., Jin, I., Lee, D. G., Kim, J., Lee, D., and Lee, W. J. (2006) An essential complementary role of NF-kappaB pathway to microbicidal oxidants in Drosophila gut immunity. EMBO J. 25, 3693–3701