Supplementary Methods

Bacterial strains, plasmids and media

The oligonucleotide primers used to construct the mutant cells are listed in Table S10 (attached at the end of Supplementary Methods). Genes, *hha*, *ydgT* and *hns* were inactivated with a one-step inactivation method[1] using primer pairs TOP679-TOP680, TOP681-TOP682 and TOP1376-TOP1377, respectively. Plasmids pKD4 or pKD3[1] were used as templates for the amplification of DNA fragments to replace the coding regions of the *hha*, *hns* and *ydgT* genes with those for kanamycin resistance (*hha* and *hns*) or chloramphenicol resistance (*ydgT*); this was done in BW25113 cells harboring the pKD46 plasmid (encoding the lambda red recombinase).[1] The *hha*::Km, *hns*::Km and *ydgT*::Cm alleles were P1 transduced into strain W3110 to establish W3110 *hns*::Km (TU03), W3110 *hha*::Km (TU04) and W3110 *ydgT*::Cm (TU11), respectively. The TU03 and TU04 strains were then transformed with *stpA*::Cm and *ydgT*::Cm alleles via P1 transduction to create W3110 *hns*::Km *stpA*::Cm (TU05) and W3110 *hha*::Km *ydgT*::Cm (TU07), respectively. To prepare the P1 lysate for *stpA*::Cm allele, strain ZEU02[2] was used.

Strain W3110 *hns*-3xflag *hha*::km *ydgT*::cm (TU01) was constructed by the P1 transduction of the *hns*::km and *stpA*::cm alleles into W3110 *hns*-3xflag (TON1897) which were created by removing the Km resistance gene from W3110 *hns*-3xflag Km (TON1816)[2] using the FLP recombinase expressed from the pCP20 plasmid[1].

To create the plasmid for expression of N-terminally 6xHis-tagged Hha, the DNA fragment encompassing the *hha* gene (except the start codon) was PCR amplified from W3110 chromosomal DNA using the TOP780-TOP781 primer pair. The resulting DNA

fragment was digested with *Sph*I and *Pst*I, and cloned between the *SphI* and *PstI* sites of the pQE80L plasmid (Qiagen, Germany). The resultant plasmid was transformed into W3110 and W3110 *hns*::Km *stpA*::Cm (TU05), to direct the expression of 6xHis-tagged Hha in the wild-type and *hns/stpA*-inactivated genetic backgrounds, respectively (TU08 and TU10, respectively). *E. coli* strains were cultivated in LB medium (10 g of Bacto Trypton and 5 g of Yeast Extract per liter) supplemented with 0.3 M NaCl (final concentration). When required, antibiotics were used at the following concentrations: ampicillin (50 μg/ml), kanamycin (50 μg/ml) or chloramphenicol (10 μg/ml).

Transcriptome analysis

W3110, W3110 *hha*::Km (TU04), W3110 *ydgT*::Cm (TU11), W3110 *hha*::Km *ydgT*::Cm (TU07), W3110 *hns*::Km (TU03), W3110 *stpA*::Cm (ZEU02) and W3110 *hns*::Km *stpA*::Cm (TU05) cells were grown in 10 ml of LB (+0.3 M NaCl) medium under aerobic conditions at 37°C until the culture reached an OD_{600} of 0.4. Then, 5 ml of culture was mixed with 10 ml of RNA Protect (Qiagen, Germany) and the cells were collected by centrifugation and stored at −80°C. Total RNA was purified from collected cells using the RNeasy mini kit according to the manufacturer's instructions (Qiagen, Germany). Synthesis of cDNA, terminal labeling, and hybridization with the oligonucleotide chip were all performed following the Affymetrix instruction manual (Affymetrix, USA). The utilized cDNA was synthesized from 10 μg of total RNA using random primers and Superscript III reverse transcriptase (Invitrogen, Norway), followed by purification using QIAquick purification columns (Qiagen, Germany) and digestion with DNaseI (GE Healthcare, UK). The cDNA fragments were labeled with biotin-ddUTP using the GeneChip DNA Labeling Reagent (Affymetrix, USA).

Hybridization with the *E. coli* genome 2.0 array, which was designed by Affymetrix based on the annotation of genes in *E. coli* strains, including *E. coli* K-12 strain (strain MG1655), was performed for 16 hr at 42°C, followed by washing, staining and scanning using the GeneChip Operating software (GCOS), all according to the manufacturer's instructions (Affymetrix, USA). Prior to calculating the transcriptional signal intensities for each gene in *E. coli* K-12, we selected the probes which were designed to analyze of the genes in *E. coli* K-12, as four sets of probes to analyze genes in *E. coli* strains (K-12[MG1655], CFT073, O157:H7-Sakai and O157:H7-EDL933) are included in the *E. coli* genome 2.0 array, and adjusted the signal intensities of the probes to confer a signal average of 500 for each array, using the GCOS software supplied by Affymetrix (USA). To remove false positives due to low signal intensities in mutant and/or wild-type cells, genes with mutant signal intensities > 100 were analyzed to extract genes that were up-regulated in mutant cells. To extract genes that were down-regulated in mutant cells, genes with wild-type signal intensities > 100 were used. Average relative ratio (the intensity in mutant cells relative to wild-type cells [$\log_2$]) and their FDR (false discovery rate) value for each gene was calculated for four different combinations using duplicate data sets for wild-type and mutant cells using the statistical program R.

ChIP-chip analysis of H-NS binding regions

H-NS binding profiles were determined using a slight modification of the previously described ChIP-chip method.[2] W3110 *hns*-3xflag Km (TON1816) and W3110 *hha*::km *ydgT*::cm *hns*-3xflag (TU01) cells were grown in 200 ml of LB (+0.3 M NaCl) medium under aerobic conditions at 37°C until the culture reached an OD_{600} of 0.4. The cultures

were then treated with formaldehyde (final concentration, 1%) for 30 min at room temperature to crosslink H-NS-Flag to the chromosomal DNA. The excess formaldehyde was quenched by incubation for 10 min with 1.5 ml of 3M glycine solution. Cells were harvested, washed with Tris-buffered saline (TBS), and lysed with lysis buffer (10 mM Tris-HCl pH 8.0, 20% sucrose, 50 mM NaCl, and 10 mM EDTA). Washed cells were suspended in 500 μl of lysis buffer containing 20 mg/ml lysozyme and incubated for 30 min at 37°C. The samples were then treated with 4 ml of IP buffer (50 mM HEPES-KOH pH 7.5, 200 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, and 5% glycerol) and phenylmethylsulfonyl fluoride (PMSF; final concentration, 1 mg/ml). The cells were sonicated (XL2020; Astrason, USA) on ice for 1 min, repeated 10 times at 1 min intervals. The disrupted cell solutions were clarified by centrifugation at 15,000 rpm for 30 min at 4°C. The supernatant (whole cell extract) was mixed with anti-FLAG antibody (Sigma-Aldrich, Germany)-coated-protein A Dynal Dynabeads (100.02; Invitrogen, Norway), which were prepared as previously described.[3] The beads were incubated at 4°C overnight with rotation, and then rinsed twice with IP buffer for 10 min with rotation at 4°C, once with IP salt buffer (IP buffer containing 500 mM NaCl), once with wash buffer (10mM Tris-HCl pH8.0, 250mM LiCl, 1mM EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate), and then once with TE (10 mM Tris-HCl pH8.0, 1 mM EDTA pH 8.0). The Flag-tagged H-NS-bound DNA fragments were released from the beads by addition of 100 μl elution buffer (250 mM Tris-HCl pH 7.5, 50 mM EDTA pH8.0, 5% SDS) followed by heating at 65°C for 20 min. Flag-tagged H-NS in whole cell extracts and immunoprecipitated DNA fractions were digested with 2 mg/ml proteinase K (Takara, Japan) at 42°C for 2 hr, followed by incubation at 65°C for 6 hr to decrosslink

H-NS-Flag and the chromosomal DNA, and to inactivate the proteinase K. Free DNA fragments in the whole cell extracts (Sup DNA) and immunoprecipitated DNA fractions (ChIP DNA) were purified with a QIAquick purification kit (Qiagen, Germany) and eluted with 100 μl of the provided elution buffer. The recovered DNA was PCR amplified as described previously.[3] Labeling of the amplified DNA fragments, hybridization with the custom-designed Affymetrix oligonucleotide tiling array, and data acquisition were all performed according to the procedure supplied by Affymetrix (USA). The utilized Affymetrix oligonucleotide tiling array was designed from the *E. coli* K-12 chromosome sequence (AP009048.1; information on the design of the probes in this tiling array has been deposited in EMBL array express under accession number A-AFFY-191). Raw data (CEL files) were processed using the Array edition of the In Silico Molecular Cloning (IMC) software (In Silico Biology, Japan), as follows. The signal intensities of mismatch probes were subtracted from those of perfect-match probes. Probes with low signal intensities (the lowest 10% of all probes) for Sup DNA and negative values for both ChIP and Sup DNA were excluded from further analysis. The signal intensities of the ChIP DNA and Sup DNA were adjusted to confer a signal average of 500. Then, to quantitatively estimate the enrichment of DNA fragments by immunoprecipitation (enrichment factor), the signal intensities of ChIP DNA were divided by those of Sup DNA.

H-NS binding regions were determined using a slight modification of the previously described method.[4] Briefly, chromosomal regions in which at least five probes at intervals of less than 50 bp yielded signal intensities above the threshold value of 3.0 were extracted as possible binding regions. Such regions deduced from two independent experiments for each strain were compared, and overlapping regions longer than 150 bp

were defined as H-NS binding regions.

ChAP-chip analysis of Hha binding regions

Hha binding profiles were determined using the previously described modified ChIP-chip method (ChAP-chip method) [4,5] with slight adjustments. W3110 pQE80Hha (TU08) and W3110 *hns*::km *stpA::*Cm pQE80Hha (TU10) cells were grown in 50 ml of LB (+0.3 M NaCl) medium with aeration at 37°C until the culture reached an OD_{600} of 0.1. Then, isopropyl β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM and the cultivation was continued to an OD_{600} of 0.4, for expression of N-terminally 6xHis-tagged Hha. The cells were then incubated with formaldehyde (final concentration, 1%) for 30 min at room temperature to crosslink Hha to the chromosomal DNA, washed with TBS (pH 7.5), and stored at −80°C. The collected cells were disrupted by sonication on ice in 3 ml of UT buffer (100 mM HEPES, 50 mM imidazole, 8 M urea, 0.5 M NaCl, 1% Triton X-100, and 10 mM β-mercaptoethanol, pH 7.4) containing a protease inhibitor cocktail (Roche, Germany). After centrifugation at 15,000 rpm for 30 min, 25 µl of Dynabeads His-Tag Isolation & Pulldown (Invitrogen, Norway) was added to the supernatant, which was then incubated for 10 min at 4°C with gentle shaking. The beads were washed five times with UT buffer, and then the bead-bound Hha complexes were eluted twice with 400 µl of elution buffer (100 mM Tris–HCl pH 7.5, 0.5M imidazole, and 1% SDS). The eluted complexes were passed through Microcon-10 (Millipore, USA), and complexes retained on the membrane were washed three times with wash buffer (50 mM Tris–HCl pH 7.5, 1% SDS, and 10 mM EDTA). Purification of DNA fragments from the whole cell extracts (Sup DNA) and affinity-purified fractions (ChAP DNA; same as ChIP DNA in

ChIP-chip experiment described above), hybridization of Sup DNA and ChAP DNA with the custom designed Affymetrix oligonucleotide tiling array, and processing the hybridization data were performed as described above.

Hha binding regions were determined as described above, except that the threshold value was set to 2.0 because the Hha binding signal intensities were lower than those of H-NS (probably due to an indirect association with chromosome DNA).

Ribosomal and transfer RNA genes and insertion sequence (IS) elements frequently show high background signals in ChIP-chip and ChAP-chip analyses, due to their high transcriptional activity and repetitive sequences. We removed these sequences from our analysis.

References

1. 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. USA*, **97**, 6640-6645.
2. Uyar, E., Kurokawa, K., Yoshimura, M., Ishikawa, S., Ogasawara, N. and Oshima, T. 2009, Differential binding profiles of StpA in wild-type and *h-ns* mutant cells: a comparative analysis of cooperative partners by chromatin immunoprecipitation-microarray analysis, *J. Bacteriol*, **191**, 2388-2391.
3. Katou, Y., Kaneshiro, K., Aburatani, H. and Shirahige, K. 2006, Genomic approach for the understanding of dynamic aspect of chromosome behavior, *Methods Enzymol.*, **409**, 389-410.

4 Chumsakul, O., Takahashi, H., Oshima, T., et al. 2011, Genome-wide binding profiles of the *Bacillus subtilis* transition state regulator AbrB and its homolog Abh reveals their interactive role in transcriptional regulation, *Nucleic Acids*

Res., **39**, 414-428.

5 Ishikawa, S., Ogura, Y., Yoshimura, M., et al. 2007, Distribution of stable DnaA-binding sites on the *Bacillus subtilis* genome detected using a modified ChIP-chip method, *DNA Res*, **14**, 155-168.

Table S10. Oligonucleotides used in this study

Primers	DNA sequence (5'→3')
TOP679	ATAAAATTCTTAATAAACAGCCGGTTATAGCTCCGAAAGCGAATT ATTTAgtgtaggctggagctgcttc [a]
TOP680	GAATCCTGCGAGTTTATCTTGTTAGAATTATTACAACCATAGGTAG AAGTcatatgaatatcctcctta
TOP681	GAAATTTTATCATCACTAATAATACCTACCCCTTTGGTATTAATTAT TTtgtgtaggctggagctgcttc
TOP682	TAATTTTAGGGGCGTTATTATAAGGCTTTTATAACCGATCGCTACTT TCTcatatgaatatcctccttag
TOP1376	AATTTAAGGCTCTATTATTACCTCAACAAACCACCCCAATATAAGT TTGAGATTACTACAtgtgtaggctggagctgcttc
TOP1377	ACCGGACAATAAAAAATCCCGCCGCTGGCGGGATTTTAAGCAAG TGCAATCTACAAAAGAcatatgaatatcctcctt
TOP780	gcggcatgc[b]TCCGAAAAACCTTTAACGA
TOP781	cgcctgcagTTAGCGAATAAATTTCCAT

a: Lower case letters represent the priming sequences for pKD4 and pKD3 (TOP679-682, TOP1376-1377). b: Underlined lower case letters indicate the recognition sequences for the restriction enzymes, *Sph*I (gcatgc) and *Pst*I (ctgcag).