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Functions of the Hha and YdgT Proteins in Transcriptional Silencing by the Nucleoid Proteins, H-NS and StpA, in *Escherichia coli*

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Abstract

The Hha and YdgT proteins are suggested to modulate the expression of horizontally acquired genes by interacting with H-NS and StpA, which play central roles in the transcriptional silencing of such genes. However, it is also possible that Hha/YdgT repress gene expression independently of H-NS/StpA, as we have not fully understood the molecular mechanism through which Hha/YdgT modulate H-NS/StpA activity. To gain further insight into the basic functions of Hha/YdgT, we analysed the impact of *hha*/*ydgT* double inactivation on the transcriptome profile of *Escherichia coli* K-12, and compared the effects with that of *hns*/*stpA* double inactivation. In addition, we examined the effects of *hha*/*ydgT* inactivation on the chromosomal binding of H-NS, and conversely the effects of *hns*/*stpA* inactivation on the chromosomal binding of Hha. Our results demonstrated that the chromosomal binding of Hha requires H-NS/StpA, and is necessary for the repression of a subset of genes in the H-NS/StpA regulon. Furthermore, the distribution of H-NS binding around Hha/YdgT-dependent and -independent genes suggests that Hha/YdgT proteins modulate formation of the H-NS/StpA-DNA complex.

Key words: *Escherichia coli*; Hha; YdgT; H-NS; StpA

1. Introduction

The H-NS protein is a major nucleoid component and is conserved amongst γ-proteobacteria. Recent studies have revealed that H-NS plays a central role in transcriptional silencing of horizontally acquired genes[1–5] by binding to the nucleation sites in AT-rich sequences and forming higher-order nucleoprotein complexes.[6–9] *Escherichia coli* and related enteric bacteria possess four H-NS homologues, such as H-NS, StpA, Hha and YdgT,[10] and their mutual interactions have been demonstrated.[11–13]

Both H-NS and StpA comprise two domains, with the N-terminal domain involved in dimerization and oligomerization and the C-terminal domain involved in DNA binding.[14] Inactivation of *hns* was found to result in de-repression of hundreds of genes in *E. coli* and *Salmonella*.[2,4,14,15] In contrast, *stpA* inactivation is not associated with any notable phenotype under standard growth conditions in *E. coli*, probably because its function is compensated by H-NS. Conversely, it has been suggested that StpA partially compensates *hns* inactivation.[16–18] Also, *stpA* transcription is induced in an *hns* mutant, and growth impairment of the *hns*/*stpA* double mutant is severer than that of the *hns* single mutant.[19,20] Recently, we reported that the StpA-binding regions on the *E. coli* K-12 chromosome essentially overlap with those of

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H-NS, and that StpA binding was reduced to one-third in the *hns* mutant, but H-NS binding was unaffected by *stpA* inactivation.[21] Thus, StpA is considered to be a molecular back-up of H-NS in *E. coli*.[13,19–21]

Hha structurally resembles the N-terminal domain of H-NS,[10] and its expression is sensitive to osmolality.[22] Hha was first identified as a repressor of the haemolysin operon (*hlyCABD*) in *E. coli* 5K.[23–25] In *Salmonella enterica*, Hha negatively regulates *hilA* and *rtsA*, each encoding activator of virulence genes.[26–29] Furthermore, the Ler regulator of the *esc* operon in *E. coli* O157:H7 is negatively regulated by Hha.[30] These virulence genes are under the control of H-NS, and Hha is generally believed to modulate the DNA-binding and nucleoid-organizing properties of H-NS.[10,24,25] Gel retardation analysis has shown that Hha binds to the regulatory regions of the *rtsA* and the *hilA* gene.[26,29] Till now, functional studies of Hha have been hampered by the presence of its paralog, YdgT.[10] Transcription of *ydgT* is induced by *hha* inactivation, while its overexpression was shown to repress the *hly* operon in the *hha* mutant,[11] suggesting that the functions of Hha and YdgT may overlap. Furthermore, simultaneous inactivation of *hha* and *ydgT* in *S. enterica* induced numerous genes located in AT-rich, horizontally acquired DNA sequences, many of which are reported to be targets of H-NS.[31,32]

Taken together, these data indicate that Hha/YdgT function with H-NS/StpA to regulate the expression of horizontally acquired genes. The lack of clear DNA-binding domains in Hha and YdgT strongly suggests that they interact with H-NS/StpA to confer their regulatory activity. However, it remains possible that Hha/YdgT may repress gene expression independently of H-NS/StpA. In addition, the molecular mechanism through which Hha/YdgT modulate the activity of H-NS/StpA in a subset of genes in the H-NS/StpA regulon remains to be clarified. Hha/YdgT are conserved in non-pathogenic *E. coli*, and Hha is reportedly involved in regulating *htrA* expression to promote survival at high temperatures,[33] and in regulating biofilm formation in *E. coli* K-12.[34]

To gain further insight into the basic function of Hha/YdgT, we compared the transcriptome profiles of *hha/ydgT* double-inactivation mutants with those of *hns/stpA* double-inactivation mutants in *E. coli* K-12. In addition, we examined the effects of *hha/ydgT* inactivation on the chromosomal binding of H-NS, and the effects of *hns/stpA* inactivation on the chromosomal binding of Hha as shown below.

2. Materials and methods

2.1. Bacterial strains, plasmids and media

The *E. coli* K-12 strains (strain W3110 and its derivatives) and plasmids used in this study are listed in Supplementary Table S1. The construction of mutant strains and the media used in this study are detailed in Supplementary Methods.

2.2. Transcriptome analysis

The detailed procedures for RNA purification, probe preparation and data acquisition using the Affymetrix *E. coli* genome 2.0 array are described in Supplementary Methods. The raw data (CEL format) from transcriptome experiments have been deposited in ArrayExpress under accession number E-MEXP-3811.

2.3. Profiling chromosome binding of H-NS and Hha

H-NS and Hha binding profiles were determined using a slight modification of the previously described ChIP-chip[21] and ChAP-chip methods.[35,36] The detailed procedures for these analysis were described in Supplementary Methods. The raw data (CEL format) from the ChIP-chip and the ChAP-chip experiments have been deposited in ArrayExpress under accession numbers E-MEXP-3812, E-MEXP-3813, respectively.

3. Results and discussion

3.1. Transcriptome analysis of hha/ydgT and hns/stpA double mutants of E. coli K-12

To avoid compensatory effects of the paralogous proteins, we created *hha/ydgT* and *hns/stpA* double mutants of *E. coli* K-12, and compared their transcriptome profiles with that of wild-type. Also, we used cells cultivated under high osmolality condition (LB medium containing 0.3 M NaCl) in which Hha regulated the expression of proteins in *E. coli*.[37] *Escherichia coli* cells were grown aerobically and the expression level of each gene was assessed using an Affymetrix *E. coli* genome 2.0 array. Up- or down-regulation in the mutant was judged by the difference in the expression level of >4- or <0.25-fold (>2- or <-2-fold in $\log_2$ scale) compared with that of wild-type, with a false discovery rate (FDR) of <0.1.

The single inactivation of *hns* had a significant impact on the transcriptome, resulting in up-regulation of 172 genes and down-regulation of one gene (Fig. 1A and Supplementary Table S2). Up-regulation of gene expression in the *hns* mutant could reflect indirect effects of down-regulation of the *hns* homologues. However, we believe that this is unlikely, since *hns* inactivation up-regulated the expression level of *stpA*, *hha* and *ydgT* by 1.98-, 1.41- and 2.03- ($\log_2$) fold, respectively, compared with that in wild-type (Supplementary Table S3). In contrast, the single inactivation of *stpA* had a far less significant effect (Fig. 1B).

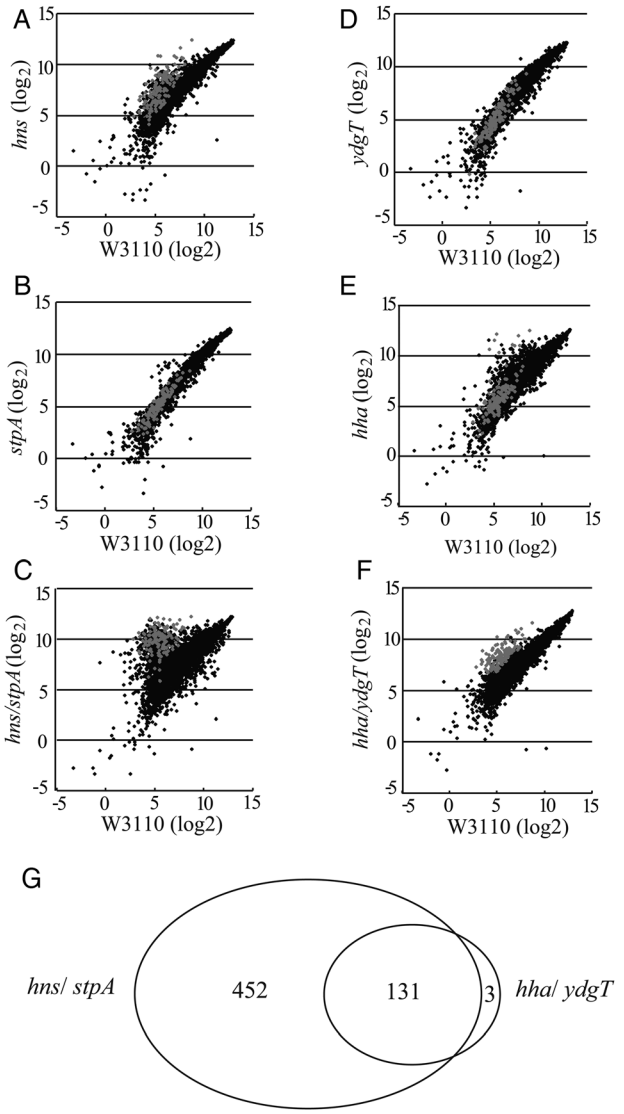

Figure 1. Transcriptome analysis of mutant cells. (**A**–**F**) Log-scale scatter plots ($\log_2$) of the transcriptional intensities of each gene in *hns* (A), *stpA* (B), *hns*/*stpA* (C), *ydgT* (D), *hha* (E) and *hha*/*ydgT* (F) mutant cells (vertical axis) compared with those in wild-type cells (horizontal axis). The average signal intensities from two independent experiments using each strain are plotted. Genes up-regulated in *hha*/*ydgT* mutant cells are shown as grey dots. (**G**) Venn diagram indicates the number of shared and unique genes up-regulated in *hha*/*ydgT* and *hns*/*stpA* mutant cells.

The *hns*/*stpA* double mutant showed further alterations in the transcriptome profile, with up-regulation of 583 genes, 167 of which were included in the 172 genes up-regulated in the *hns* single mutant, and down-regulation of 86 genes compared with wild-type (Fig. 1C and Supplementary Table S4). Notably, the up-regulation of *hha* and *ydgT* seen in the *hns* mutant also occurred in the *hns*/*stpA* double mutant (Supplementary Table S3), indicating that the up-regulation observed in the *hns*/*stpA* mutant was not caused by the down-regulation of *hha* and/or *ydgT*. Of the genes up-regulated by *hns* inactivation, 69% (119 of 172) have been proposed to be horizontally acquired.[38,39] Similarly, 62% (363 of 583) of the genes up-regulated in the double mutant were horizontally acquired.

The single inactivation of *ydgT* had a much less effect, up-regulating only one gene (*yhjX*) and down-regulating six genes (*yfiD*, *sraA*, *pdhR*, *tke1*, *yifE* and *yejG*; Fig. 1D). In contrast, single *hha* inactivation up-regulated 113 genes and down-regulated 8 genes (Fig. 1E and Supplementary Table S5). In addition, it moderately up-regulated *ydgT* [1.48- ($\log_2$) fold], but did not cause significant changes in *hns* and *stpA* (FDR $>$ 0.1; Supplementary Table S3), indicating that the observed up-regulation was a direct consequence of the *hha* inactivation. The genes up-regulated in the *hha* mutant included those related to osmotic (e.g. *osmY*, *kdpA* and *kdpC*) and carbon-starvation (e.g. *csiD* and *rmf*) stresses, which is consistent with the previous finding that genes related to these stresses were induced in *hha* mutant cells.[34] The *hha*/*ydgT* double inactivation showed transcriptional alterations in a similar number of genes, with 134 genes up-regulated and 5 genes down-regulated (Fig. 1F and Supplementary Table S6). Consistent with the previous observation in *S. enterica*,[32] most of the genes that were up-regulated in *hha*/*ydgT*-inactivated cells (108 of 134) are believed to be horizontally acquired (Supplementary Table S6). No statistically significant change was detected in the expression of *hns* or *stpA* in the *hha*/*ydgT* mutant (Supplementary Table S3).

Only 12 genes were commonly up-regulated in the *hha* and *hha*/*ydgT* mutants (Fig. 1E, grey dots; Supplementary Tables S5 and S6). Furthermore, although most of the genes that were up-regulated in the *hha*/*ydgT* mutant were also up-regulated in the *hns*/*stpA* mutant (see below), only 47 of the 113 genes that were up-regulated in the *hha* single mutant were up-regulated in the *hns*/*stpA* mutant (Supplementary Tables S4 and S5). These results suggest that the mechanism responsible for the up-regulation of genes in the single *hha* mutant differs from that in the *hha*/*ydgT* double mutant, although we did not further explore the molecular mechanism causing this phenotype in the *hha* mutant.

In contrast to the altered transcriptome profile in the *hha* mutant, 131 of the 134 genes that were up-regulated in the *hha*/*ydgT* double mutant were also up-regulated in the *hns*/*stpA* double mutant (Fig. 1G, Venn diagram; Fig. 1C, grey dots; Supplementary Tables S4 and S6). Of the three genes that were significantly up-regulated only in the *hha*/*ydgT* mutant, two were probably also up-regulated in the *hns*/*stpA* mutant, though at

a low signal intensity (*fimZ*) or low induction level (*yhjA*). Thus, only *ycgX* appears to be regulated by Hha/YdgT and not by H-NS/StpA. Taken together, these results indicate that Hha and YdgT are additionally required for the repression of a subset of genes that are repressed by H-NS and StpA in *E. coli* K-12.

3.2. *H-NS binding profiles are not altered by* hha/ydgT *inactivation*

To investigate whether Hha/YdgT modulate the DNA-binding activity of H-NS/StpA, we examined effects of *hha/ydgT* inactivation on the chromosomal binding of H-NS. To this end, we introduced the *hha/ydgT* mutation into a strain in which H-NS was tagged with 3× Flag and placed under the control of the native promoter.[5,21] Wild-type and *hha/ydgT* mutant cells expressing H-NS-3× Flag were cultivated in LB (+0.3 M NaCl) medium, formaldehyde treated to crosslink H-NS-Flag with the chromosomal DNA, and then harvested and subjected to immunoprecipitation of H-NS–DNA complexes. The DNA fragments co-purified with H-NS were mapped onto the chromosome by using a custom Affymetrix tiling array. As shown in Figure 2A, scatter plots of the H-NS binding signals in both strains showed a genome-wide positive correlation (correlation coefficient = 0.91). H-NS-binding regions, 417 regions in wild-type and 530 in the *hha/ydgT* mutant, were reproducibly observed in duplicate ChIP-chip analyses (Supplementary Fig. S1 and Table S7). Moreover, 96% (400 of 417) of the binding regions in wild-type overlapped with those in the *hha/ydgT* mutant (Supplementary Table S7).

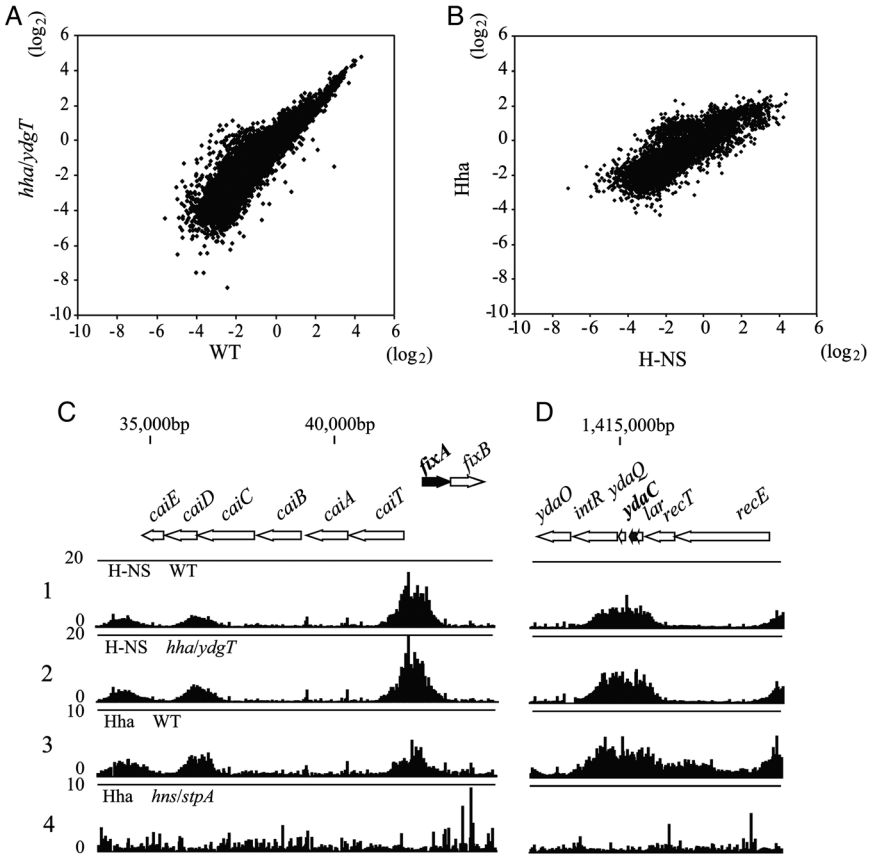

Figure 2. Impact of *hha/ydgT* double inactivation on H-NS bindings and the genome-wide correlation of H-NS and Hha bindings. (**A** and **B**) Log scatter plots (log_2) of the average signal intensities of H-NS binding signals from two independent experiments using wild-type (W3110) and *hha/ydgT* mutant cells (A), and H-NS binding signals in wild-type (W3110) cells and Hha binding signals in W3110 pQEHha cells (B). (**C** and **D**) Typical examples of H-NS-binding profiles in W3110 (lane 1) and W3110 *hha*::Km *ydgT*::Cm (lane 2), and Hha-binding profiles in W3110 pQE80Hha (lane 3) and W3110 *hns*::Km *stpA*::Cm pQE80Hha (lane 4) are shown with the binding signal of each probe mapped to the corresponding position in the *E. coli* chromosome. The binding intensity (vertical axis) was determined as the relative ratio of the signal intensity for the hybridization of labelled DNA fragments prepared from the ChIP (ChAP) versus Sup fractions in each experiment. The Hha binding signals were low throughout the genome of *hns/stpA* mutant cells (lane 4), and the background spike signals were enhanced when we conferred a signal average of 500 during signal intensity normalization prior to calculating the binding intensity. Shown are the H-NS and Hha binding profiles in the vicinity of a gene (*fixA*; black thick arrow) that was up-regulated only in *hns/stpA* mutant cells (C), or the H-NS- and Hha-binding profiles in the vicinity to a gene (*ydaC*) that was up-regulated in both *hns/stpA* and *hha/ydgT* mutant cells (D).

Although many of the H-NS-binding regions found in wild-type were segmented in the *hha/ydgT* mutant and H-NS-binding signals were slightly stronger in the *hha/ydgT* mutant than in wild-type, 84% (444 of 530) of the regions overlapped with those in wild-type (Supplementary Table S7) and weak binding signals (below the threshold) were observed in wild-type in the remaining regions as well (Supplementary Fig. S1). In addition, there was no significant difference between the effect of Hha/YdgT inactivation on H-NS binding in the vicinity of genes up-regulated only in *hns/stpA* mutant (Fig. 2C) and of those up-regulated in both *hha/ydgT* and *hns/stpA* mutants (Fig. 2D). Thus, we concluded that the *hha/ydgT* mutation had little effect on the DNA-binding property of H-NS.

3.3. *Hha associates with the chromosome in an H-NS/StpA-dependent manner*

To examine whether Hha/YdgT interact with target genes independently of H-NS/StpA, we analysed Hha binding on the chromosome in wild-type and the *hns/stpA* mutant. As the expression level of Hha from the native promoter was expected to be low, to obtain clear chromosomal binding profile by the ChIP-chip analysis,[12] cells of wild-type and the *hns/stpA* mutant expressing 6× histidine-tagged Hha (6 × His-Hha) from a multi-copy plasmid were cultivated in LB (+0.3 M NaCl) medium and subjected to a modified ChIP-chip analysis (ChAP-chip analysis) in which His-tagged Hha–DNA complexes were affinity purified using nickel affinity resin. Western blot analysis of cell extracts with an anti-His antibody indicated that the expression level of 6 × His-Hha in wild-type and *hns/stpA* mutant cells was similar (data not shown). Therefore, the binding signals of Hha consistently overlapped with those of H-NS in wild-type (Fig. 2C and D lane 3; Supplementary Fig. S1), while the Hha-binding signals seen in wild-type disappeared in the *hns/stpA* mutant (Fig. 2C and D lane 4; Supplementary Fig. S1). Scatter plots of Hha- and H-NS-binding signals (Fig. 2B) support the genome-wide positive correlation of H-NS- and Hha-binding signals (correlation coefficient = 0.77). Seventy-two per cent (302 of 417) of the H-NS-binding regions were found to overlap with the Hha-binding regions, and conversely 74% (352 of 476) of the Hha-binding regions overlapped with the H-NS-binding regions (Supplementary Table S8). This lesser level of overlap between the Hha- and H-NS-binding regions may be due to the low binding signals against the high background seen in the ChAP-chip analysis of Hha binding, which may reflect its indirect interaction with the chromosomal DNA. It should also be noted that Hha binding exhibited no preference towards the genes up-regulated in *hns/stpA* and those up-regulated in both *hha/ydgT* and *hns/stpA* under the experimental conditions (compare lane 3 in panels Fig. 2C and D). However, it is possible that the reduced amount of Hha/YdgT expressed from the native promoter form a limited amount of H-NS/StpA-Hha/YdgT complexes that recognize the specific regions. Further analysis is needed to clarify the possibility.

Only five regions were found to be possible Hha-binding regions in the *hns/stpA* mutant (Supplementary Table S9), although the signals observed in the *lacI* and *hha* genes may probably be due to contamination from the multi-copy plasmid harbouring *lacI* and *hha*. Therefore, H-NS/StpA-independent chromosomal binding of Hha appear to be possible only in the intergenic region of *ygjI–ygjH* and the coding region of *fecA*. We concluded that the binding of Hha to the chromosome essentially depends on H-NS/StpA.

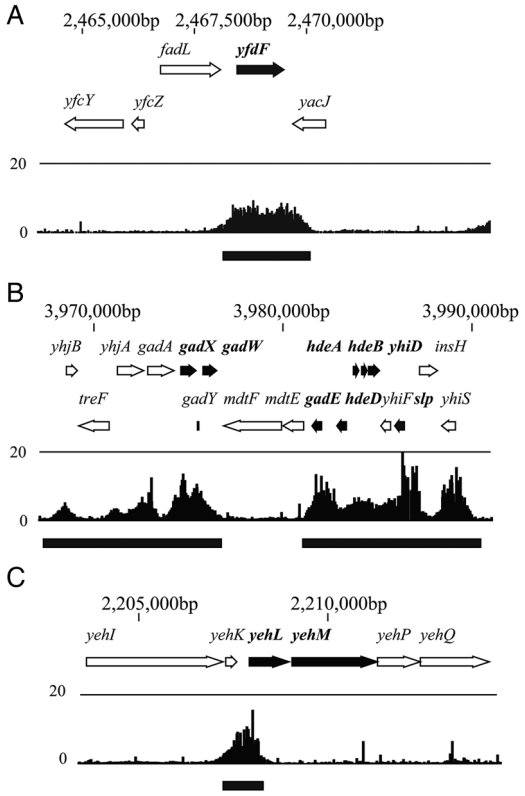

Figure 3. H-NS binding signals for genes up-regulated in the *hns/stpA* mutant alone or in both *hns/stpA* and *hha/ydgT* mutants. Figures represent the typical H-NS binding signals classified as 'coding (single)' [in the vicinity of gene (*yfdF*; the thick black arrow) that were up-regulated in both *hns/stpA* and *hha/ydgT* mutants] (**A**), 'coding (multiple)' [in the vicinity of genes (*yhiD*, *hdeB*, *hdeA*, *hdeD*, *slp*, *gadE*, *gadW* and *gadX*) that were up-regulated in both *hns/stpA* and *hha/ydgT* mutants] (**B**), and 'intergenic' [in the vicinity of genes (*yehL* and *yehM*) that were up-regulated only in *hns/stpA* mutants] (**C**). The H-NS-binding regions (concatenated) are shown by black lines at the bottom of the figures.

3.4. *Hha/YdgT support transcriptional repression by H-NS/StpA bound to the coding sequences*

It has been suggested that the binding of H-NS is biased to intergenic regions, where H-NS plays a regulatory role, while its binding to coding regions may be linked to chromosome organization in *E. coli*.[40] Our inspection of H-NS-binding signals in and around the genes that are co-repressed by Hha/YdgT and H-NS/StpA suggest that the coding sequences of Hha/YdgT-dependent genes tend to be bound by H-NS (Fig. 3A). Furthermore, such H-NS binding often extended into multiple coding sequences (Fig. 3B). In contrast, the H-NS binding seemed to be localized in the intergenic regions of the genes exhibiting Hha/YdgT-independent up-regulation (Fig. 3C).

To perform genome-wide evaluation of these phenomena, we visually inspected the H-NS-binding regions in wild-type into two classes: those localized to intergenic regions ('intergenic') and those extending to coding sequences ('coding') by concatenating the H-NS-binding regions with the corresponding intergenic regions or the (successive) coding sequences. A half of the concatenated H-NS-binding regions was classified as 'intergenic', as their peaks were located at intergenic sequences (Supplementary Fig. S2A and B), while the remaining half was classified as 'coding' (106 regions; Table 1), as their peaks were located at coding sequences or their H-NS-binding signals evenly covered coding sequences (Supplementary Fig. S2C and D). About a half of the 'coding' extended into multiple coding sequences (Supplementary Fig. S2E and F; Table 1). Ten regions were classified as both 'coding' and 'intergenic', as they included the intergenic sequences of one gene and the coding sequences of another gene(s) (for example, see Supplementary Fig. S3D).

We then examined the H-NS-binding regions in and around the genes that were up-regulated in the *hns/stpA* mutant, and found that those regions were classified as 73 'intergenic' and 89 'coding' (Table 1). Subsequently, we evaluated the relationship between the localization of H-NS-binding signals and

Table 1. Number of H-NS-binding regions in 'intergenic' and 'coding' sequences

Location of H-NS-binding regions	Number of regions (un)associated with H-NS/StpA-repressed genes				Total	
	Unassociated		Associated			
Localized to intergenic regions	33		73		106	
Covering coding sequences[a]	17		89		106	
	Single	Multiple	Single	Multiple	Single	Multiple
	15	2	27	62	42	64
Total	50		162		212	

[a]H-NS-binding regions covering the coding sequence(s) of single and multiple gene(s) are indicated as 'Single' and 'Multiple', respectively.

Table 2. The Hha/YdgT-dependent and -independent genes up-regulated in the *hns/stpA* mutant, and their distribution in and around the 'intergenic' and 'coding' H-NS-binding regions

Genes up-regulated in *hns/stpA* mutant[a]	Genes with intergenic H-NS binding	Genes with coding H-NS binding		Genes without H-NS-binding regions[b]
Hha/YdgT-independent (436 genes)	136 (31%)[c]	243 (55%)		57 (13%)
		Single	Multiple	
		25 (6%)	218 (50%)	
Hha/YdgT-dependent (122 genes)	11 (9%)[d]	108 (88%)		3 (2%)
		Single	Multiple	
		12 (10%)	96 (79%)	

[a]Twenty-five genes that were found to be up-regulated in the *hns/stpA* mutant cells (as detected by transcriptome analysis using the *E. coli* genome 2.0 array) were not annotated in the genome sequence of *E. coli* K-12 strain W3110, which was used to design the custom tiling chip for ChIP-chip analysis. We evaluated the correlation of transcriptome alteration and H-NS binding profiles according to the W3110 annotation, and analysed the locations of the 436 genes up-regulated only in the *hns/stpA* mutant and the 122 genes up-regulated in both the *hns/stpA* and *hha/ydgT* mutants. Genes located in or around the H-NS-binding regions were classified as 'coding' or 'intergenic', respectively.

[b]Sum of the genes that were not located in or around the H-NS-binding regions but showed up-regulation in the *hns/stpA* mutant alone or in both the *hns/stpA* and *hha/ydgT* mutants.

[c]Number and percent with respect to the 436 genes up-regulated only in the *hns/stpA* mutant.

[d]Number and percent with respect to the 122 genes up-regulated in both the *hns/stpA* and *hha/ydgT* mutants.

the ability of the genes to undergo Hha/YdgT-dependent or -independent repression by H-NS/StpA (Table 2). A majority of the genes up-regulated in both *hha/ydgT* and *hns/stpA* mutants was localized in and around H-NS-binding regions classified as 'coding' (108 genes; 88%; Supplementary Fig. S3), including those covering multiple coding sequences. Hha/YdgT-independent genes were more enriched (136 genes; 31%) in the genes that were regulated by binding of H-NS/StpA to their intergenic regions than Hha/YdgT-dependent genes (11 genes; 9%), although many Hha/YdgT-independent genes were also found in 'coding' H-NS-binding regions (243 genes; 55%). These results suggest that Hha/YdgT are required for efficient transcriptional repression by H-NS/StpA bound to coding sequences.

3.5. *Possible mechanism of transcriptional repression by H-NS and Hha*

A number of mechanisms have been proposed to explain the inhibition of transcriptional initiation or elongation by H-NS.[10,14,41,42] H-NS binds to two distinct sites and forms a bridge structure to block the binding of RNA polymerase (RNAP) to a promoter[43] or trap RNAP in an open complex at a promoter, thereby inhibiting transcriptional elongation.[44,45] Others have shown that the binding of H-NS upstream of a regulatory region interferes with either promoter clearance or progression of RNAP,[46] and H-NS has been shown to constrain local DNA topology to regulate the promoter activity of supercoiling-sensitive genes.[47–49] Mutations impairing the ability of H-NS and its homologue of *Pseudomonas aeruginosa* termed MvaT to form higher-order oligomers have been shown to reduce their abilities to repress transcription of the *E. coli proU* and *P. aeruginosa cupA* fimbria genes.[18,50,51] The formation of a DNA–protein filament through cooperative polymerization of MvaT or H-NS along DNA and of protein bridges to constrain DNA loops through the interaction of the DNA–protein filaments were suggested to be important for transcriptional repression.[7,52] The direct interaction of Hha and C-terminally truncated H-NS (H-NS_{64}) was previously demonstrated by NMR and fluorescence anisotropy.[53,54] In the absence of Hha, H-NS_{64} formed only dimers even at a high concentration; in the presence of Hha, however, H-NS_{64} formed high-molecular-mass Hha-(H-NS_{64}) hetero-oligomers.[53] We speculate that Hha enhances the higher-order oligomerization of H-NS/StpA and contributes to the formation of DNA–protein filaments and DNA loops, which often include the coding sequences of target genes. The 5.5 protein of phage T7 binds to the oligomerization domain of H-NS to inhibit the oligomerization of H-NS and the repression of more than 200 genes, although the DNA binding of H-NS is not abolished.[55] Up-regulation of genes observed in the *hha/ydgT* mutant might be similar to that caused by the T7 5.5 protein. Even though H-NS binding is not greatly affected in *hha/ydgT* mutant cells, the oligomerization of H-NS/StpA might be reduced, resulting in the up-regulation of the genes that are normally repressed by oligomerized H-NS/StpA. However, we do not yet fully understand the mechanism underlying the enhancement of H-NS/StpA oligomerization by Hha/YdgT and the repression of transcription by oligomerized H-NS/StpA. Future work is needed to elucidate the co-repression activity of Hha/YdgT and H-NS/StpA.

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