

# Genome-Wide Identification of H-NS-Controlled, Temperature-Regulated Genes in *Escherichia coli* K-12<sup>∇†</sup>

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**DNA microarrays demonstrate that H-NS controls 69% of the temperature regulated genes in *Escherichia coli* K-12. H-NS is shown to be a common regulator of multiple iron and other nutrient acquisition systems preferentially expressed at 37°C and of general stress response, biofilm formation, and cold shock genes highly expressed at 23°C.**

Temperature is one of the many signals bacteria use as a cue for modulating gene expression. Genome-wide studies have provided evidence that human body temperature has a broad role in regulating gene expression patterns that facilitate effective host colonization (3, 11, 18, 21, 26, 28, 31, 33, 39), while low growth temperatures influence the expression of genes required for adaptation to vector hosts, aquatic environments, or biomedically relevant ambient room settings (3, 11, 18, 20, 21, 26, 28, 31, 33, 34, 40).

The histone-like nucleoid structuring (H-NS) protein, conserved among gram-negative bacteria, regulates the transcription of many environmentally responsive genes, implicating this regulator in bacterial adaptation to changing conditions, including temperature (reviewed in references 4 and 24). In *Escherichia coli*, temperature regulation and H-NS have primarily been studied in the control of specific operons related to virulence, including fimbriae, toxins, and pathogenicity island-associated genes. (1, 7, 8, 10, 14, 15, 19, 22, 25, 35–37, 41, 42).

In this study, the genome-wide role of H-NS in controlling temperature-regulated genes was investigated in *E. coli* K-12. Gene expression ratios of an *hns651* mutant strain grown at 37 and 23°C were determined and subsequently compared to those ratios obtained with the wild-type strain in previous microarray studies (39, 40) to identify H-NS-mediated changes as a function of temperature. The strain used (DL1947) contains an insertion in *hns* that abrogates expression of the H-NS protein but is otherwise identical to the wild-type strain (DL1504) (9, 37). The *hns651* mutant was grown as described previously (39) at 37 and 23°C in M9 glycerol medium with aeration, and RNA was harvested in exponential phase at 9 to 11 generations of growth after inoculation, thus reflecting genes whose expression is differentially maintained over long-term growth at 37 and 23°C. cDNAs were cohybridized to microarray slides containing oligonucleotides representing all

of the genes of *E. coli* K-12; H-NS-controlled genes are those in which the ratio of gene expression measured at the two temperatures in the *hns651* mutant differed from that measured in the wild-type strain. This change in the expression ratio indicates a role for H-NS in regulation by altering transcription at either one or both temperatures. While comparison of these microarray data sets obtained at different time points did not offer gene expression ratios (mutant/wild type) at each temperature, it allowed the sensitive detection of thermally H-NS-regulated genes.

**H-NS controls more than two-thirds of the temperature-regulated genes in *E. coli* K-12 but also many nonthermoregulated genes.** Of the 122 thermoregulated genes with increased expression at 37°C compared to 23°C, 73 were identified as being H-NS controlled (Table 1; see Table S1 in the supplemental material). For 60 of these genes, the absence of H-NS reduced or eliminated a thermoregulatory response whereas 13 genes showed a statistically significant expansion of differential expression between 37 and 23°C. Several genes identified by this strategy (*srlAD*, *cysPWU*, *garLPR*, *fes*, and *cirA*) were previously shown to be thermoregulated and H-NS controlled by quantitative reverse transcription-PCR (qRT-PCR) (39), supporting the validity of the approach.

Of the 297 genes more highly expressed at 23°C in wild-type bacteria, 215 are regulated by H-NS. Of these genes, 179 showed a reduced or total loss of a thermoregulatory response between 37 and 23°C in the *hns651* mutant whereas 36 demonstrated a statistically significant expanded thermoregulatory differential (Table 1; see Table S2 in the supplemental material).

For genes that did not show a thermoregulatory response in the wild-type strain, a large number demonstrated a statistically significant difference in expression between 37 and 23°C in the *hns651* mutant. Increased expression in the *hns651* mutant at 37°C was observed for 308 genes (Table 1; see Table S3 in the supplemental material), whereas 264 showed increased expression at 23°C (Table 1; see Table S4 in the supplemental material), indicating that H-NS contributes to the regulation of many genes that are unaffected by growth temperature.

**Comparison to other genome-wide analyses reveals the direct targets of H-NS binding and that H-NS regulates many genes common to both pathogenic and nonpathogenic *E. coli* strains.** Oshima et al. characterized approximately 250 H-NS binding sites within the *E. coli* K-12 genome by chromatin

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TABLE 1. Characterization of H-NS regulated genes<sup>a</sup>

Description	Total no. of genes	No. with temp differential in <i>hms651</i> mutant		Highly represented categories of genes (no. of genes)
		Decreased/absent	Increased	
Thermoregulated with higher expression at 37°C in wild type <sup>b</sup>	73	60	13	Iron utilization (10), carbohydrate transport and metabolism (14), amino acid transport and metabolism (16)
Thermoregulated with higher expression at 23°C in wild type <sup>c</sup>	215	179	36	RpoS controlled (89), biofilm (19), cold shock (17), unknown function (121)
Nonthermoregulated in wild type, higher expression at 37°C in <i>hms651</i> mutant <sup>d</sup>	308	NA <sup>f</sup>	307	Amino acid transport and metabolism (38), translation (39)
Nonthermoregulated in wild type, higher expression at 23°C in <i>hms651</i> mutant <sup>e</sup>	264	NA	264	Evenly distributed among many categories

<sup>a</sup> H-NS-controlled genes were defined as those in which the ratio of gene expression levels at the two temperatures in the *hms651* mutant differed by  $\geq 0.5$  from that found in the wild-type strain.

<sup>b</sup> See Table S1 in the supplemental material.

<sup>c</sup> See Table S2 in the supplemental material.

<sup>d</sup> See Table S3 in the supplemental material.

<sup>e</sup> See Table S4 in the supplemental material.

<sup>f</sup> NA, not applicable.

immunoprecipitation (ChIP)-chip analyses (27). Binding sites either 5' upstream or within their coding sequences mapped to genes we identified as being H-NS regulated, including 19% of the genes (14 genes) more highly expressed at 37°C (see Table S1 in the supplemental material) and 33% of the genes (70 genes) more highly expressed at 23°C (see Table S2 in the supplemental material), suggesting that the transcriptional effects at these promoters are due to direct binding of the H-NS protein. Among those genes that are not temperature regulated but demonstrate H-NS regulation, there was also a significant overlap with the H-NS binding site database. Approximately 10% of the genes (34 genes) more highly expressed at 37°C (see Table S3 in the supplemental material) and 45% of the genes (120 genes) more highly expressed at 23°C (see Table S4 in the supplemental material) in the *hms651* mutant have H-NS binding sites associated with the operons that contain them. Interestingly, there are similar numbers of genes that are direct targets of H-NS, regardless of whether or not they are thermoregulated.

In comparison to other genome-wide studies assessing H-NS control of transcription at a single temperature, 157 H-NS-regulated genes identified in our study overlap those in uropathogenic strain 536 (23) and 47 genes in *E. coli* K-12 (13) (see Tables S1 to S4 in the supplemental material), encompassing both thermoregulated and nonthermoregulated genes.

**H-NS regulates 60% of the genes more highly expressed at 37°C and is a common regulator of multiple iron uptake systems in *E. coli*.** Our previous studies demonstrated that the mammalian host temperature (37°C) serves to increase and maintain 122 genes at a higher steady-state level of expression compared to 23°C (39), and the results presented here show that H-NS contributes to the regulation of 60% of these genes. The majority of these genes are involved in nutrient uptake—amino acid transport and metabolism (16), carbohydrate transport and metabolism (14), and inorganic ion transport and metabolism (11)—and their higher expression at 37°C may be particularly beneficial to host colonization.

Of the genes with increased expression at 37°C, 10 iron utilization genes in seven different operons are temperature

regulated and controlled by H-NS. To corroborate the microarray results and demonstrate how H-NS specifically contributes to gene expression, relative mRNA levels at 37 and 23°C in the wild-type and *hms651* mutant strains were analyzed by qRT-PCR as previously described (39) (Table 2). Representative genes within the ferric enterobactin (*fep*), ferric citrate (*fec*), ferrichrome (*fhu*), and ferrous (*feo*) systems were investigated to determine if H-NS control is broadly applicable to iron uptake. For genes in the *fep*, *fec*, and *fhu* systems, expression of the iron utilization genes in the wild-type strain is reduced at 23°C compared to that at 37°C, confirming temperature as a common regulatory cue for these genes (Table 2). In the *hms651* mutant, the expression of all of these genes is statistically significantly decreased at 37°C compared to that in the wild-type strain, indicating a positive role for H-NS. At 23°C, the effect of H-NS on expression is variable in these systems, with some being unaltered by the *hms651* mutation (*fecA*, *fecI*, *fhuE*) whereas others are reduced (*fepC*, *fepD*, *fhuA*) in comparison to the wild-type strain, indicating a positive regulatory role. Previous studies in our laboratory show a similar trend for two other iron acquisition genes, *cirA* and *fes* (39).

In contrast to the other iron uptake systems, the *feoA* gene appears not to be temperature regulated and reveals equivalent expression levels at both 37 and 23°C (Table 2). The introduction of the *hms651* mutation led to significantly increased expression at both 37 and 23°C in comparison to that in the wild-type strain (Table 2). It is interesting that H-NS binding sites are associated with the *feoAB* operon based on ChIP-chip analyses (27), correlating to the only operon we tested at which H-NS acts purely as a repressor of transcription. With the exception of *fepE*, no binding sites for H-NS were found to be associated with any of the iron utilization genes within the *fep*/*ent*, *fhu*, or *fec* system, arguing that the effect on gene expression in these operons by H-NS is likely indirect.

Fur (ferric uptake regulator) is an obvious candidate for an intermediate regulator targeted by H-NS. Transcription of the genes in these systems is responsive to the iron concentration,

TABLE 2. Iron utilization gene mRNA levels at 37 and 23°C in wild-type and *hns651* mutant strains<sup>a</sup>

Gene	Wild type		<i>hns651</i> mutant		37/23°C ratio		<i>hns651</i> /wild-type ratio	
	37°C	23°C	37°C	23°C	Wild type	<i>hns651</i> mutant	37°C	23°C
<i>fepC</i>	<b>1.0</b> (0.9–1.1)	<b>0.6</b> (0.5–0.6)	<b>0.7</b> (0.4–1.2)	<b>0.3</b> (0.2–0.4)	<b>1.7</b>	<b>2.3</b>	<b>0.7</b>	<b>0.5</b>
<i>fepD</i>	<b>1.0</b> (0.7–1.5)	<b>0.5</b> (0.3–0.9)	<b>0.5</b> (0.2–0.9)	<b>0.3</b> (0.2–0.4)	<b>2.0</b>	<b>1.7</b>	<b>0.5</b>	<b>0.6</b>
<i>fecA</i>	<b>1.0</b> (0.8–1.3)	<b>0.2</b> (0.2–0.3)	<b>0.6</b> (0.3–1.0)	<b>0.2</b> (0.2–0.3)	<b>5.0</b>	<b>2.5</b>	<b>0.5</b>	<b>1.0</b>
<i>fecI</i>	<b>1.0</b> (0.6–1.7)	<b>0.4</b> (0.2–0.6)	<b>0.7</b> (0.5–1.2)	<b>0.3</b> (0.2–0.4)	<b>2.5</b>	<b>2.3</b>	<b>0.7</b>	<b>0.8</b>
<i>fhuA</i>	<b>1.0</b> (0.6–1.6)	<b>0.4</b> (0.3–0.6)	<b>0.2</b> (0.1–0.4)	<b>0.2</b> (0.2–0.4)	<b>2.5</b>	<b>1.0</b>	<b>0.2</b>	<b>0.5</b>
<i>fhuE</i>	<b>1.0</b> (0.7–1.5)	<b>0.6</b> (0.4–0.9)	<b>0.4</b> (0.3–0.6)	<b>0.6</b> (0.4–0.8)	<b>1.7</b>	<b>0.7</b>	<b>0.4</b>	<b>1.0</b>
<i>feoA</i>	<b>1.0</b> (0.7–1.4)	<b>0.9</b> (0.6–1.3)	<b>3.0</b> (2.3–3.9)	<b>5.0</b> (3.7–6.8)	<b>1.1</b>	<b>0.6</b>	<b>3.0</b>	<b>5.6</b>
<i>fur</i>	<b>1.0</b> (0.9–1.1)	<b>0.9</b> (0.6–1.3)	<b>0.6</b> (0.4–0.8)	<b>0.6</b> (0.4–0.8)	<b>1.1</b>	<b>1.0</b>	<b>0.6</b>	<b>0.7</b>

<sup>a</sup> Gene expression levels were measured by qRT-PCR. For each gene, the average expression level is in bold and is relative to the level measured at 37°C in wild-type strain DL1504. Differences in gene expression were determined to be statistically significant ( $P < 0.05$ ) by two-way analysis of variance. All of the data shown were determined to be statistically significantly different from those for the wild-type strain at 37°C. Values in parentheses are standard deviations based on the results of three independent experiments.

showing high expression under iron-depleted conditions but repressed by the transcriptional regulator Fur when iron is present (reviewed in references 6 and 12). We hypothesized that H-NS might repress *fur* transcription, resulting in increased transcription of this repressor in an *hns651* mutant strain. However, studies of *fur* expression demonstrated that *fur* was not temperature regulated and that the *hns651* mutation led to slightly decreased, rather than increased, *fur* mRNA levels at both temperatures (Table 2). While we cannot discount that the *hns651* mutation might influence Fur protein levels or activity, this result raises the intriguing possibility that H-NS controls an unknown common intermediate that controls the *fec*, *fhu*, and *fep* systems. These findings may be particularly relevant to pathogenesis, given that these iron uptake systems are conserved in several strains of pathogenic *E. coli* and that H-NS is known to control genes within the uropathogenic *E. coli* yersiniabactin and salmochelin iron uptake systems (23).

**H-NS regulates 72% of the genes more highly expressed at 23°C and regulates RpoS and DsrA levels to modulate RpoS-dependent gene expression.** Our previous studies demonstrated that a low growth temperature of 23°C serves to increase and maintain 297 genes at a higher steady-state level of expression than does a growth temperature of 37°C (39), and the results presented here show that H-NS contributes to the regulation of 72% of these genes. Eighty-nine were RpoS-controlled genes associated with the general stress response, 19 genes were associated with biofilm development, and 17 were associated with the cold shock response (40), suggesting a strong linkage between these response mechanism pathways and H-NS (Table 1; see Table S2 in the supplemental material). More than 50% of the genes that are temperature regulated and H-NS controlled are of uncharacterized function, indicating there is much to be learned about adaptation to growth at low temperature.

Because more than 40% of the genes with increased expression at low temperature are RpoS and H-NS controlled, we investigated the effect of the *hns651* mutation on transcription, both of the regulators themselves (RpoS and DsrA) and of a representative subset of genes whose expression at 23°C is known to be RpoS and DsrA dependent (40). RpoS levels are increased at low temperature by the small regulatory RNA

DsrA (32), which alters *rpoS* mRNA secondary structure to allow more efficient *rpoS* translation (reviewed in references 16 and 30) and subsequent increased transcription of RpoS-dependent genes. In addition to the *rpoS* mRNA, DsrA has also been shown to target *hns* mRNA for degradation, subsequently decreasing H-NS levels (17). Thus, DsrA is thought to both increase RpoS and decrease H-NS protein levels to allow transcription of the general stress response genes at low temperature. In our studies of the wild-type strain, expression of *rpoS* and *dsrA* is increased at 23°C, consistent with previous studies indicating that their transcription is temperature regulated (Table 3) (29, 32). In the *hns651* mutant strain, *rpoS* levels are not significantly altered, whereas levels of *dsrA* are significantly increased at both 37 and 23°C. To test whether the *hns651* mutation leads to increased RpoS, protein levels were analyzed at 37 and 23°C in the wild-type and *hns651* mutant strains by Western blotting (data not shown). While RpoS is present in both the wild-type and *hns651* mutant strains at 23°C, it is only present at 37°C in the *hns651* mutant and not in the wild-type strain. This result is consistent with the hypothesis that H-NS contributes to the thermoregulatory control of RpoS expression. It should be noted that separate studies demonstrated that in an *hns* mutant both the half-life of DsrA (2) and the stability of RpoS are increased (43) at 37°C.

mRNA levels were measured at 37 and 23°C for a subset of genes whose expression at 23°C is RpoS and DsrA dependent in the wild-type and *hns651* mutant strains (38). Genes associated with biofilm formation (*bolA*, *csgA*, and *nhaR*) and the cold shock response (*otsA*, *yceP*, and *ycgZ*) and genes with unknown function (*ymdA* and *yhiM*) were tested. At 37 and 23°C, *otsA*, *yhiM*, and *yceP* expression in the *hns651* mutant equals or exceeds that observed at 23°C in the wild-type strain, indicating an exclusively repressive regulatory role for H-NS in the control of these operons at both 37 and 23°C. However, for *bolA*, *ycgZ*, *ymdA*, *csgA*, and *nhaR*, while the *hns651* mutation leads to increased mRNA levels at 37°C in comparison to the wild-type strain at 37°C, they do not reach the maximal levels observed at 23°C. With the exception of *bolA*, this was true also at 23°C, where the *hns651* mutant showed reduced expression levels of these genes in comparison to those in the wild-type strain at the same temperature. According to ChIP-chip analyses (27), the operons containing these genes are all direct

TABLE 3. RpoS- and DsrA-dependent gene mRNA levels at 37 and 23°C in wild-type and *hns651* mutant strains<sup>a</sup>

Gene(s)	Wild type		<i>hns651</i> mutant		23/37°C ratio		<i>hns651</i> /wild-type ratio	
	37°C	23°C	37°C	23°C	Wild type	<i>hns651</i> mutant	37°C	23°C
<i>rpoS</i>	<b>1.0</b> (0.9–1.1)	<b>1.9</b> (1.5–2.4)	<b>0.7</b> (0.5–1.0)	<b>2.5</b> (1.8–3.4)	<b>1.9</b>	<b>3.6</b>	<b>0.7</b>	<b>1.3</b>
<i>dsrA</i>	<b>1.0</b> (0.7–1.4)	<b>5.6</b> (3.5–8.8)	<b>4.6</b> (2.9–7.2)	<b>11.7</b> (8.7–15.8)	<b>5.6</b>	<b>2.5</b>	<b>4.6</b>	<b>2.1</b>
<i>bolA</i>	<b>1.0</b> (0.8–1.2)	<b>5.5</b> (3.8–7.9)	<b>2.9</b> (2.2–3.9)	<b>12.8</b> (10.8–15.3)	<b>5.5</b>	<b>4.4</b>	<b>2.9</b>	<b>2.3</b>
<i>csgA</i>	<b>1.0</b> (0.7–1.4)	<b>745.7</b> (610.8–910.4)	<b>10.9</b> (6.5–18.0)	<b>135.8</b> (97.1–190)	<b>746</b>	<b>12.5</b>	<b>10.9</b>	<b>0.2</b>
<i>nhaR</i>	<b>1.0</b> (0.9–1.2)	<b>3.9</b> (2.9–5.2)	<b>2.5</b> (1.7–3.8)	<b>2.8</b> (2.0–4.0)	<b>3.9</b>	<b>1.1</b>	<b>2.5</b>	<b>0.7</b>
<i>otsA</i>	<b>1.0</b> (0.8–1.3)	<b>3.0</b> (2.4–3.9)	<b>11.6</b> (9.4–14.3)	<b>13.2</b> (9.3–18.8)	<b>3.0</b>	<b>1.1</b>	<b>11.6</b>	<b>4.4</b>
<i>yceP/bssS</i>	<b>1.0</b> (0.9–1.1)	<b>3.6</b> (2.7–4.7)	<b>3.2</b> (2.3–4.4)	<b>13.6</b> (4.8–39.2)	<b>3.6</b>	<b>4.3</b>	<b>3.2</b>	<b>3.8</b>
<i>yegZ</i>	<b>1.0</b> (0.9–1.1)	<b>21.1</b> (17.8–24.9)	<b>5.1</b> (3.7–7.0)	<b>16.1</b> (13.6–19.0)	<b>21.1</b>	<b>3.2</b>	<b>5.1</b>	<b>0.8</b>
<i>yhiM</i>	<b>1.0</b> (0.6–1.7)	<b>7.1</b> (4.3–11.8)	<b>38.3</b> (22.9–63.8)	<b>225.0</b> (149.4–338.8)	<b>7.1</b>	<b>5.9</b>	<b>38.3</b>	<b>31.7</b>
<i>ymdA</i>	<b>1.0</b> (0.6–1.7)	<b>30.5</b> (15.1–61.9)	<b>1.8</b> (1.0–3.2)	<b>6.1</b> (4.1–9.1)	<b>30.5</b>	<b>3.4</b>	<b>1.8</b>	<b>0.2</b>

<sup>a</sup> Gene expression levels were measured by qRT-PCR. For each gene, the average expression level is in bold and is relative to the level measured at 37°C in wild-type strain DL1504. Values in parentheses are standard deviations based on the results of three independent experiments.

targets of H-NS, suggesting that derepression at the nonpermissive temperature (37°C) in the *hns651* mutant may be due, at least in part, to the loss of H-NS binding at these operons. Concomitant with this, *hns651* also leads to increased RpoS levels at 37°C that could initiate the transcription of RpoS-dependent genes. However, for several of the genes tested, the effects on transcription cannot be attributed only to these two mechanisms as the absence of H-NS does not result in levels of expression at 37°C that match those seen at 23°C in the wild-type strain. The observation that these genes do not reach maximal levels in an *hns651* mutant at either temperature argues that there are likely additional, indirect, effects of the *hns651* mutant on other factors required for efficient transcription of these operons. Thus, no simple single regulatory role of H-NS can be invoked for the control of RpoS- and DsrA-dependent genes.

**H-NS regulates gene expression at both temperatures.** While H-NS controls a majority of thermoregulated genes, our study, along with others (reviewed in reference 24), indicates that H-NS frequently regulates gene expression at both temperatures rather than fitting an “all-or-nothing” model where it functions only at one temperature. Comparison of the *hns651* mutant and wild-type expression ratios (Tables 2 and 3) of several iron utilization genes (*fepC*, *fepD*, *fecI*, *fhuA*, and *fur*) demonstrated similarly reduced transcription at both temperatures, while *bolA*, *yceP*, and *yhiM* were similarly derepressed by the *hns651* mutation at 37 and 23°C, indicating an equivalent role for H-NS at both temperatures that may serve to modulate basal levels of transcription. In addition, the large number of nonthermoregulated genes controlled by H-NS (572) questions the characterization of H-NS as a molecular thermometer. However, H-NS-mediated repression is greater at 37°C than at 23°C for *dsrA* and *otsA*; the opposite is true for *feoA*, where repression by H-NS is greater at 23°C. Thus, at other operons, H-NS functions at both temperatures but with a more pronounced effect at one temperature. While they were less frequent, some genes (*fecA*, *fhuE*) showed expression patterns where the effect of H-NS was only observed at one temperature (37°C). Thus, our findings agree with other studies indicating that H-NS is present and influences transcription at both high and low temperatures, although in some cases (e.g.,

*dsrA* and *feo*) it appears that its repressive effect can be differentially modulated by temperature.

The authors of a recent study with *Salmonella* postulate that temperature and H-NS play an important role in niche-specific programming of virulence gene expression (5). In this model, temperature functions as an important top-level cue that prevents the production of virulence factors in nonhost environments, even when other environmental cues might lead to their expression. Our studies and others indicate that temperature and H-NS have a broader effect beyond virulence gene expression, acting to fine-tune and regulate a number of genes, allowing efficient colonization of the host and enabling appropriate adaptation to external environmental temperatures.

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