

RESEARCH COMMUNICATION

Widespread suppression of intragenic transcription initiation by H-NS

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Widespread intragenic transcription initiation has been observed in many species. Here we show that the *Escherichia coli* *ehxCABD* operon contains numerous intragenic promoters in both sense and antisense orientations. Transcription from these promoters is silenced by the histone-like nucleoid structuring (H-NS) protein. On a genome-wide scale, we show that 46% of H-NS-suppressed transcripts in *E. coli* are intragenic in origin. Furthermore, many intergenic promoters repressed by H-NS are for noncoding RNAs (ncRNAs). Thus, a major overlooked function of H-NS is to prevent transcription of spurious RNA. Our data provide a molecular description for the toxicity of horizontally acquired DNA and explain how this is counteracted by H-NS.

Supplemental material is available for this article.

Received November 6, 2013; revised version accepted December 17, 2013.

Pervasive transcription is a phenomenon ubiquitous across the three domains of life (Dornenburg et al. 2010). It is defined by an undercurrent of noncanonical transcripts within the primary transcriptome (Clark et al. 2011). In bacteria, such transcripts arise from normal promoter elements but are conspicuous because they are noncoding, not delineated by gene boundaries, and sometimes antisense (Peters et al. 2012). Sites of pervasive transcription are poorly conserved. Hence, even between closely related bacteria, patterns of pervasive transcription differ (Raghavan et al. 2012). This has led to speculation that pervasive transcription is a consequence of the evolution of protein-coding genes (Peters et al. 2012; Raghavan et al. 2012). It is likely that pervasive transcription is suppressed, as spurious RNA synthesis is potentially catastrophic. Synthesis, stability,

and translation of mRNA may be perturbed (Dornenburg et al. 2010), and formation of R loops (DNA:RNA hybrids) can destabilize genome integrity (Gowrishankar and Harinarayanan 2004).

In *Escherichia coli*, there are two major sources of pervasive transcription: inefficient termination of RNA synthesis at gene boundaries and spurious transcription initiation in genes (Dornenburg et al. 2010; Peters et al. 2012). Attention has turned to elucidating mechanisms that suppress these phenomena. As a first step, Peters et al. (2012) identified Rho and the cofactor NusG as suppressors of pervasive antisense transcription. In their model, Rho acts via two pathways. First, Rho ensures termination of transcripts at gene boundaries, preventing “leaky” transcription of downstream genes. Second, Rho terminates antisense transcripts arising from promoters in genes. Thus, on a global scale, noncanonical transcripts are targeted for termination. However, since most transcription is regulated at the level of initiation (Browning and Busby 2004), additional mechanisms to suppress pervasive transcription are likely.

The histone-like nucleoid structuring (H-NS) protein is a DNA folding factor. It is abundant in bacteria and influences transcription (Dillon and Dorman 2010). Nucleoprotein containing H-NS is structurally diverse; H-NS can form bridged or filamentous DNA complexes (Dame et al. 2006; Liu et al. 2010). Two mechanisms for transcriptional repression by H-NS have been proposed: (1) exclusion, in which H-NS masks RNA polymerase (RNAP)-binding sites (Lim et al. 2012), and (2) trapping, in which H-NS ensnares initiating RNAP in DNA loops (Dame et al. 2002). H-NS may also slow elongation, leading to increased Rho-dependent termination (Peters et al. 2012). Chromatin immunoprecipitation (ChIP) has been used to map binding of H-NS across bacterial chromosomes (Grainger et al. 2006; Lucchini et al. 2006; Navarre et al. 2006; Oshima et al. 2006; Kahramanoglou et al. 2011). These studies show that H-NS targets A/T-rich DNA, a distinguishing feature of horizontally acquired genes. Consequently, H-NS has been proposed to facilitate genome evolution by silencing transcription of foreign DNA (Navarre et al. 2006). A central tenet of this model is that foreign DNA is toxic. However, a molecular explanation for this toxicity and how H-NS counteracts this is lacking.

Here, we describe widespread suppression of intragenic transcription initiation in bacteria. We show that this is a major function of H-NS. First, we show that H-NS represses transcription from many intragenic promoters within the A/T-rich *ehxCABD* operon of *E. coli* O157:H7. Second, we show that most H-NS-repressed promoters genome-wide in *E. coli* K-12 are for noncoding RNAs (ncRNAs), with many promoters located inside genes. Third, we show that suppression of widespread intragenic transcription is a likely function of H-NS analogs in diverse bacterial species.

[*Keywords:* H-NS; nucleoid; pervasive transcription]

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Article published online ahead of print. Article and publication date are online at <http://www.genesdev.org/cgi/doi/10.1101/gad.234336.113>. Freely available online through the *Genes & Development* Open Access option.

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Results and Discussion

The enterohemorrhagic E. coli O157:H7 ehxCABD operon contains many intragenic promoters

The *ehxCABD* operon is a well-characterized H-NS target (Li et al. 2008; Rogers et al. 2009; Iyoda et al. 2011). Our previous analysis of *ehxCABD* suggested the existence of intragenic promoters (Singh and Grainger 2013). We began here by scanning the operon for sequences that resemble promoter -10 elements. As a control, we applied the same analysis to the *E. coli* O157:H7 and K-12 genomes. For *ehxCABD*, our analysis identified 95 putative intragenic -10 elements, an approximately fourfold enrichment over the genome averages for both *E. coli* strains. To test for function, we cloned the 95 sequences, each on a 62-base-pair (bp) DNA fragment, upstream of *lacZ* in the reporter plasmid pRW50 (Supplemental Fig. S1). In some cases, predicted -10 elements overlapped. Hence, we were able to clone the 95 sequences on 83 DNA fragments. Twenty of the fragments stimulated β -galactosidase activity twofold or more above background (Fig. 1). We selected five of the 20 active constructs representing sense and antisense promoters with a wide range of activities (labeled a–e in Fig. 1) and examined the effects of disrupting the predicted -10 element. In all cases, this resulted in a significant decrease in β -galactosidase activity (Supplemental Fig. S2).

Direct repression of intragenic promoters in the ehxCABD operon by H-NS

Transcription of *ehxCABD* is well characterized as being repressed by H-NS (Li et al. 2008; Rogers et al. 2009; Iyoda et al. 2011). Despite this, H-NS is unable to block transcription from the canonical *ehxCABD* promoter (Singh and Grainger 2013). We hypothesized that H-NS acts via the intragenic *ehxCABD* promoters. To test this, we measured β -galactosidase activity in wild-type and Δhns

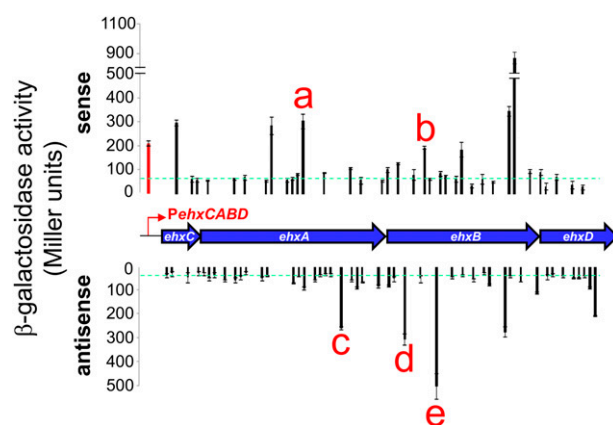


Figure 1. The *E. coli ehxCABD* operon contains many intragenic promoters. β -Galactosidase activity driven by short intragenic *ehxCABD* promoter fragments in strain JCB387. The *top* chart shows the activity of promoters that drive sense transcription, and the *bottom* chart shows the activity of promoters that drive antisense transcription. Each data point is aligned with the corresponding section of the *ehxCABD* operon. Data points labeled a–e correspond to promoters used in further experiments. The red bar represents the canonical promoter for *ehxCABD*. Background activity (empty pRW50) is shown by a green line.

cells containing different sections of *ehxCABD* fused to *lacZ* in plasmid pRW50. First, we examined a large, 5429-bp section of *ehxCABD* lacking the canonical upstream promoter. Transcription initiating in this coding DNA, which is repressed fivefold by H-NS, must be due to intragenic promoters (Fig. 2A, panel i). Sequential removal of upstream sequences reduces repression by H-NS (Fig. 2A, panel ii). Similarly, transcription from promoters a–e was unaffected by H-NS in the context of the 62-bp fragments (Fig. 2A, panel iii). We conclude that H-NS represses transcription from intragenic *ehxCABD* promoters, but repression requires extensive flanking DNA. Note that the sequence of the flanking DNA is also crucial because, when flanked by plasmid DNA, the 62-bp *ehxCABD* fragments were not subject to repression by H-NS.

Given that H-NS repressed intragenic *ehxCABD* promoters only in the context of larger *ehxCABD* fragments, we predicted that H-NS would bind tightly throughout the operon but weakly to small fragments in pRW50. Consistent with this, H-NS forms bridged and filamentous complexes with DNA that are more stable than isolated H-NS-binding events (Bouffartigues et al. 2007). Hence, we measured binding of H-NS to promoters a–e in the context of the large (5429-bp) and short (62-bp) fragments of *ehxCABD* cloned in pRW50. ChIP was used to measure binding of H-NS to the different targets *in vivo*. Figure 2B shows PCR analysis of DNA immunoprecipitated with anti-H-NS. Control experiments analyzing DNA recovered from mock immunoprecipitations are also shown. As predicted, loci a–e bind H-NS in the context of the large *ehxCABD* fragment (Fig. 2B, top panel). Conversely, H-NS did not bind the same loci when they were isolated from the rest of the operon (Fig. 2B, bottom panel). No enrichment of the control chromosomal *yabN* locus, which is not bound by H-NS, was detected in any immunoprecipitate (Supplemental Fig. S3). In complementary experiments, we measured binding of H-NS to the same DNA fragments *in vitro* using electrophoretic mobility shift assays (EMSA). H-NS bound to the 5429-bp *ehxCABD* fragment but not the short intragenic promoter fragments (Fig. 2C).

To confirm direct H-NS-mediated repression of intragenic *ehxCABD* promoters, we recapitulated the phenomenon *in vitro*. Two DNA fragments were used as templates for *in vitro* transcription: a 7435-bp DNA fragment containing the entire *ehxCABD* operon and upstream region (Fig. 2D, lanes 3–9) and a 6435-bp fragment lacking the upstream region and canonical promoter (Fig. 2D, lanes 10–16). RNAP catalyzed the synthesis of multiple transcripts of varying size from each template. Consistent with a previous report (Singh and Grainger 2013), transcription from the canonical *ehxCABD* promoter was unaffected by the addition of H-NS, even at high concentrations (7312-nucleotides [nt] RNA) (Fig. 2D, lanes 3–9). Conversely, synthesis of shorter transcripts, ranging from <100 nt to thousands of nucleotides in length, was dramatically reduced by increasing concentrations of H-NS (Fig. 2D, lanes 4–9, 11–16). The similarity of transcript profiles synthesized from the two templates confirms that the short RNAs arise from internal initiation events and not from stalled transcription from the canonical promoter.

Widespread repression of intragenic promoters by H-NS

To determine whether H-NS suppresses intragenic transcription at other loci, we investigated the effect of H-NS

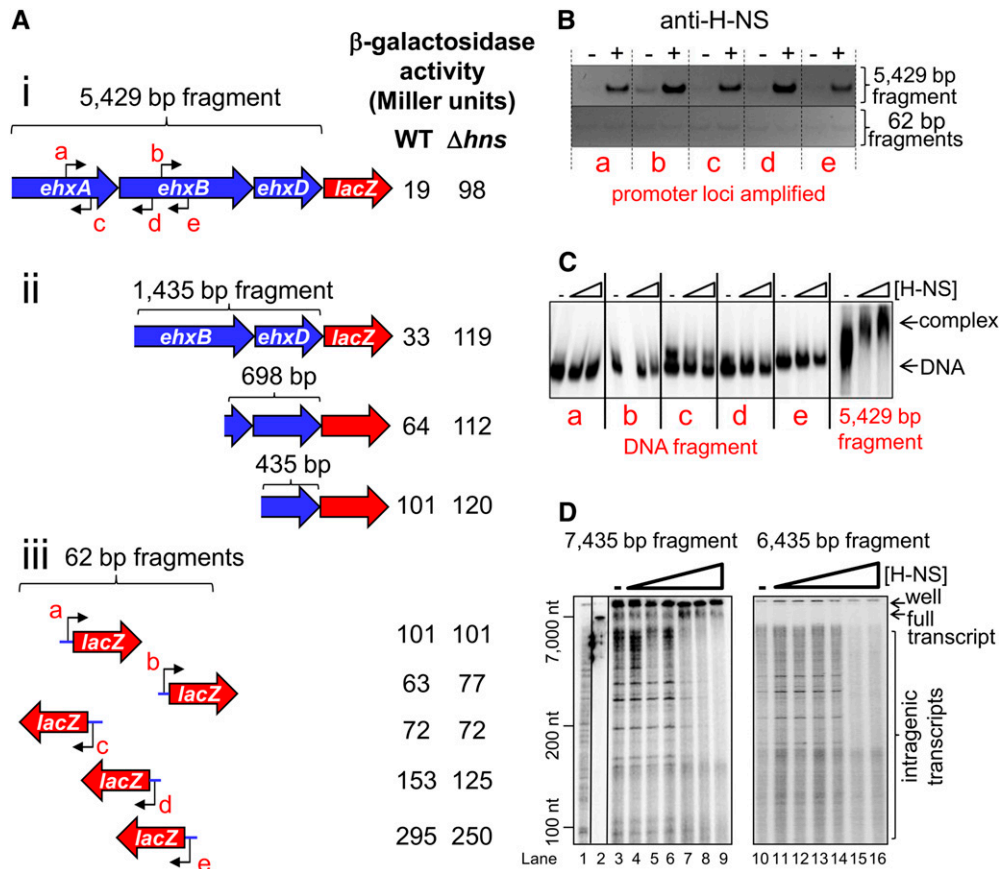


Figure 2. H-NS suppresses intragenic *ehxCABD* transcription in vivo. (A) H-NS suppresses intragenic *ehxCABD* transcription in vivo. β -Galactosidase activity stimulated by *ehxCABD* fragments fused to *lacZ* in plasmid pRW50. Intragenic promoters a–e are labeled. β -Galactosidase activity was measured in MG1655 and the Δhns derivative. Background β -galactosidase activity, obtained with empty pRW50 vector, was subtracted from the values shown. Experiments were done in triplicate, and the standard deviation was always <10% of the mean. (B) H-NS binds promoters within the *ehxCABD* operon in vivo. ChIP-PCR was used to measure binding of H-NS at intragenic *ehxCABD* loci cloned in pRW50. PCR products were generated with primers to detect the five intragenic *ehxCABD* promoters, a–e. (C) H-NS binds promoters within the *ehxCABD* operon in vitro. Electrophoretic mobility shift assays with either small (62-bp) or large (5429-bp) fragments of *ehxCABD*. The different small DNA fragments are labeled a–e. H-NS was added at a concentration of 4 μ M or 8 μ M. (D) H-NS suppresses intragenic *ehxCABD* transcription in vitro. A run-off in vitro transcription assay using DNA templates consisting of the whole *ehxCABD* operon with (7435 bp) and without (6435 bp) the upstream regulatory region. The gel was calibrated with a sequencing reaction (lane 1) and the radiolabeled DNA template (lane 2). RNAP was used at a concentration of 200 nM (lanes 3–16), and H-NS was added at concentrations of 150, 300, 600, 750, 1500, and 3000 nM (lanes 4–9,11–16).

on genome-wide transcription initiation in *E. coli* K-12. We chose *E. coli* K-12 because (1) H-NS has been studied most extensively in this strain, (2) H-NS in *E. coli* K-12 is 100% identical to that in *E. coli* O157:H7, and (3) high-resolution genome-wide binding data for H-NS, generated using ChIP sequencing (ChIP-seq), are available. As a starting point, we determined association of σ^{70} with the *E. coli* K-12 genome in wild-type and Δhns cells using ChIP-seq. Thus, we identified the location of promoter-bound RNAP and the level of occupancy (Supplemental Table S1). In parallel, we identified transcription start sites (TSSs) genome-wide in wild-type and Δhns cells using a modified RNA sequencing (RNA-seq) approach (Singh and Wade 2014). This method also determines the relative abundance of RNAs associated with each TSS (“TSS strength”). Together, these data identify sites of transcription initiation with high confidence and nucleotide resolution. Visual inspection of the data revealed many promoters repressed by H-NS (Fig. 3A). These promoters typically correspond to regions of high H-NS binding (Kahramanoglou et al. 2011). Repression was

reflected by increases in both σ^{70} occupancy and TSS strength in the Δhns mutant. Many of the H-NS-repressed promoters are located in genes.

We selected σ^{70} ChIP-seq peaks that collocated with TSSs in the Δhns mutant. Thus, we identified 668 promoters with associated information for σ^{70} occupancy, TSS strength, and TSS orientation (Supplemental Table S1). We divided the promoters into “low H-NS” (508 promoters) and “high H-NS” (160 promoters) categories based on the level of H-NS binding. For each promoter, σ^{70} binding or TSS strength was compared in Δhns and wild-type cells. The result of the comparison is in Figure 3, B (for σ^{70} binding) and C (for TSS strength). In the scatter plots, each data point represents one of the 668 promoters. Consistent with H-NS-mediated repression, association of σ^{70} with promoters in high H-NS regions was significantly higher in Δhns cells than in wild-type cells (Mann Whitney U test, normal approximation, $P = 1.4 \times 10^{-23}$) (Fig. 3B). Similarly, for high H-NS regions, TSS strength was significantly higher in Δhns cells (Mann Whitney U test, normal approximation, $P = 2.7 \times 10^{-10}$) (Fig. 3C). In

contrast, for low H-NS regions, neither σ^{70} binding nor TSS strength was significantly different in Δhns and wild-type cells (Mann Whitney U test, normal approximation, $P = 0.29$ for σ^{70} occupancy and $P = 0.23$ for TSS strength) (Fig. 3B,C). Data for all 668 promoters are summarized in Figure 3, D (σ^{70} binding) and E (TSS strength). As expected, the mean wild-type/ Δhns ratio for both σ^{70} binding and TSS strength is close to 1.0 in low H-NS regions (Fig. 3D,E, blue box). Conversely, the equivalent ratio is 0.2 or 0.3 for high H-NS regions (Figs. 3D,E, first red box). Taken together, our data indicate that high H-NS occupancy reduces the binding of initiating RNAP and represses transcription, consistent with previous studies (Dillon and Dorman 2010).

We next examined the position of the 668 promoters with respect to genes. Promoters in high H-NS regions are far more likely to be inside genes (46% of promoters) than promoters in low H-NS regions (19% of promoters; Fisher's exact test, $P = 2.0 \times 10^{-11}$). However, an intragenic or intergenic location for a promoter does not fully describe its genomic context. For example, an intergenic promoter may be orientated opposite to surrounding genes and unable to drive mRNA transcription. Conversely, an intragenic promoter may be close to a correctly orientated gene, as is the case for many known mRNA promoters (Keseler et al. 2013). Hence, we next determined the distance between each of the 668 promoters and the nearest properly orientated gene. These distances represent the length of potential mRNA 5' untranslated regions (UTRs). Of the 5' UTRs reported by RegulonDB, 95% are <227 nt in length (Salgado et al. 2013). However, only 41% of potential 5' UTRs in high H-NS regions are <227 nt in

length. This is significantly lower than in low H-NS regions, where 80% of mRNA 5' UTRs are <227 nt in length (Mann Whitney U test, normal approximation, $P = 2.4 \times 10^{-21}$) (Fig. 3F). We conclude that most promoters in regions bound by H-NS, including 31% of intergenic promoters (Fig. 3G, dark-green sector of right pie chart), likely drive synthesis of spurious ncRNAs (i.e., ncRNAs lacking function). However, we do not exclude the possibility that a small number of such promoters drive synthesis of an annotated regulatory RNA or mRNA. The genomic context of promoters in high and low H-NS regions is summarized in Figure 3G. Interestingly, while both intragenic and intergenic promoters in high H-NS regions are repressed by H-NS, repression of intragenic promoters is significantly greater (Mann Whitney U test, normal approxima-

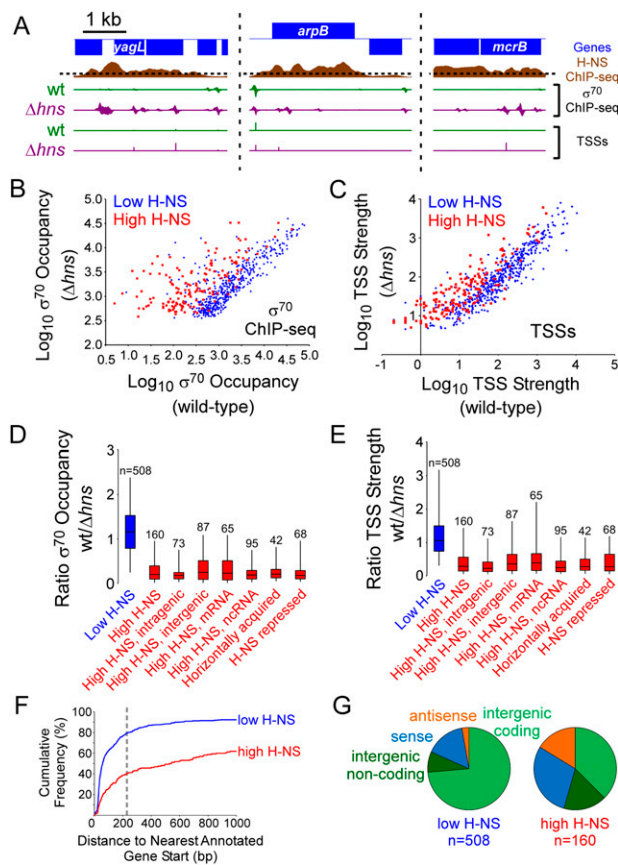


Figure 3. H-NS suppresses hundreds of intragenic promoters genome-wide. (A) Examples of promoters repressed by H-NS. Three genomic regions are shown. Data for H-NS occupancy are shown by the brown graph. The dashed line indicates the cutoff separating H-NS "high" and "low" regions. σ^{70} ChIP-seq data from wild-type cells are shown in green, and data from Δhns cells are shown in purple. TSS scores (sequence read density for a given TSS) from wild-type (green) and Δhns (purple) cells are also shown. The TSS score is shown only for TSSs identified in the Δhns mutant that were collocated with a σ^{70} ChIP-seq peak. The scale for H-NS ChIP-seq data is the same in all three panels. The scales for σ^{70} ChIP-seq and TSS data are different in each panel but consistent within each panel. (B) H-NS reduces binding of σ^{70} at many promoters. Scatter plot showing ChIP-seq σ^{70} occupancy for wild-type and Δhns cells. Each data point represents a σ^{70} ChIP-seq peak from the Δhns mutant that was collocated with a TSS. Blue and red data points are for TSSs in low and high H-NS regions, respectively. (C) H-NS reduces transcription from many promoters. Scatter plot showing TSS strength for wild-type and Δhns cells. Each data point represents a TSS from the Δhns mutant that was collocated with a σ^{70} ChIP-seq peak. Blue and red data points are for TSSs in low and high H-NS regions, respectively. (D) H-NS occludes σ^{70} from promoters inside genes, especially those that are horizontally acquired or have been described as being repressed by H-NS. Box plot showing the distribution of the ratio of σ^{70} occupancy in wild-type/ Δhns cells, as determined using ChIP-seq, for selected groups of promoters (σ^{70} ChIP-seq peaks collocated with TSSs). Boxes indicate the 25th–75th percentile. Horizontal lines indicate the median value. The whiskers indicate the 5th–95th percentile. The number of contributing promoters is indicated. Categories of promoters tested were (1) promoters in high H-NS regions, (2) promoters in low H-NS regions, (3) intragenic promoters in high H-NS regions, (4) intergenic promoters in high H-NS regions, (5) promoters in high H-NS regions that are <227 bp from the nearest appropriately orientated annotated gene start (mRNA), (6) promoters in high H-NS regions that are ≥ 227 bp from the nearest appropriately orientated annotated gene start (ncRNA), (7) promoters in horizontally acquired genes, and (8) promoters in genes described previously as being repressed by H-NS. (E) H-NS represses transcription from promoters inside genes, especially those that are horizontally acquired or have been described as being repressed by H-NS. Box plot showing the distribution of the ratio of TSS strength in wild-type/ Δhns cells (TSSs collocated with σ^{70} ChIP-seq peaks). Boxes indicate the 25th–75th percentile. Horizontal lines indicate the median value. The whiskers indicate the 5th–95th percentile. The number of contributing promoters is indicated. The categories of promoters tested match those for D. (F) Promoters in H-NS regions are often far from gene starts. Cumulative frequency distribution for the distance of TSSs (from ChIP-seq peak/TSS pairs) from the nearest appropriately orientated, annotated gene start for TSSs in low H-NS (blue line) and high H-NS (red line) regions. For a distance, x , on the X-axis, the value on the Y-axis indicates the proportion of TSSs that are $\leq x$ bp from the nearest appropriately orientated, annotated gene start. The dashed line indicates 227 bp. (G) Promoters in H-NS regions are often intragenic and/or drive transcription of ncRNAs. Pie charts showing the distribution of TSS location relative to genes in Δhns cells.

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tion, $P = 0.03$ for σ^{70} occupancy and $P = 0.01$ for TSS strength) (Fig. 3D,E, cf. the first three red data points). Thus, intragenic promoters are more likely to be repressed and are more strongly repressed by H-NS. Importantly, intragenic promoters in H-NS-bound regions are indistinguishable from all other promoters with respect to DNA sequence (Supplemental Fig. S4).

We next compared the position of H-NS-repressed promoters with horizontally acquired genes in the known H-NS regulon. Strikingly, intragenic promoters repressed by H-NS align with both horizontally acquired DNA and genes proposed to be repressed by H-NS (Fig. 3D,E, final two data points; Supplemental Table S1; Garcia-Vallve et al. 2003; Kahramanoglou et al. 2011;). We note that previous studies used microarray-based approaches unable to distinguish canonical mRNAs from ncRNAs. We conclude that many previous observations of repression by H-NS are in fact due to repression of promoters inside genes.

Binding of H-NS analogs coincides with predicted intragenic promoters in diverse bacterial species

MvaT and Lsr2 are functional analogs of H-NS in *Pseudomonas* spp. and *Mycobacterium* spp., respectively. For both proteins, ChIP-chip has been used to measure binding across the cognate genome (Castang et al. 2008; Gordon et al. 2010). Fortuitously, promoters for primary σ factors do not vary greatly between these species and *E. coli* (Potvin et al. 2008; Sachdeva et al. 2010). Hence, we were able to use the same approach used for the *E. coli* genomes to search for -10 hexamers. We then compared the position of these putative -10 hexamers with binding of MvaT/Lsr2. Putative promoters were enriched approximately fivefold in MvaT-bound (χ^2 test, $P = 8.9 \times 10^{-9}$) and Lsr2-bound (χ^2 test, $P = 5.5 \times 10^{-4}$) regions. Furthermore, most putative promoters in MvaT- and Lsr2-bound regions were located in genes (58% for *Pseudomonas aeruginosa* and 78% for *Mycobacterium tuberculosis*). Thus, MvaT and Lsr2 are likely to be suppressors of intragenic transcription.

Conclusions

H-NS has been proposed to facilitate genome evolution by binding horizontally acquired DNA. Previous studies have concluded that H-NS silences such genes solely by repressing canonical transcription (Fig. 4A). In contrast, we show that the primary function of H-NS is to silence spurious transcription of ncRNAs, often inside the genes previously described as being H-NS-repressed (Fig. 4B). Hence, the fitness defect of deleting *hns* is likely due in part to increased spurious transcription. We suggest that, by suppressing intragenic transcription, H-NS prevents misappropriation of cellular resources, the formation of dangerous nucleic acid structures, and transcription interference. We note that the two models for H-NS function shown in Figure 4 are indistinguishable using DNA microarray-based approaches that cannot resolve canonical mRNAs and spurious ncRNAs. Thus, while we have identified a largely new set of H-NS regulated promoters, these promoters coincide with the existing H-NS regulon. Previous bioinformatic screens predicted that H-NS-bound genes contain promoters (Tutukina et al. 2007). Such genes are often horizontally acquired, are A/T-rich, and have many Tpa steps common to both H-NS targets

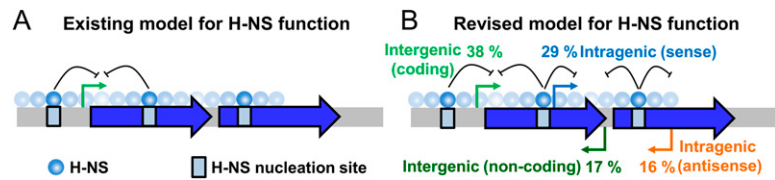


Figure 4. A new model for silencing at horizontally acquired genes by H-NS.

and promoter -10 elements (Bouffartigues et al. 2007; Singh et al. 2011). Hence, these genes are ideal candidates for recognition by H-NS or its functional analogs and for spurious RNAP binding. In such regions, high-affinity canonical promoters and transcription factors likely play a key role, along with H-NS, in facilitating specific transcription.

Materials and methods

Strains and plasmids

E. coli JCB387 Δ nir Δ lac and MG1655 have been described (Page et al. 1990; Keseler et al. 2013). The Δ hns MG1655 derivative (Fig. 2) was provided by Ding Jin. Plasmid pRW50 was described by Lodge et al. (1992). The 7435-bp *ehxCABD* fragment was synthesized by DNA2.0 and contained silent mutations to remove restriction sites to facilitate cloning. The ~ 62 -bp *ehxCABD* fragments were generated with oligonucleotides (Supplemental Table S2). A small number of fragments contained more than one putative promoter and were thus larger (up to 80 bp). Mutations in -10 elements were introduced with oligonucleotide derivatives (Supplemental Table S2). Mutations were numbered on the assumption that -10 elements are 7 bp upstream of a TSS. The Δ hns::kan^R MG1655 derivative used for ChIP-seq was constructed by P1 transduction of Δ hns::kan^R from BW25113 Δ hns::kan^R (Baba et al. 2006) into MG1655. Derivatives of BW25113 [Δ (*araD-araB*)567, Δ (*lacZ4787::rrnB-3*), λ -, *rph-1*, Δ (*rhaD-rhaB*)568, and *hsdR514*] were used for TSS mapping. Unmarked BW25113 Δ rppH was constructed by removal of *kan*^R from BW25113 Δ rppH::kan^R using FLP recombinase from plasmid pCP20 (Datsenko and Wanner 2000). BW25113 Δ rppH Δ hns::kan^R was constructed by P1 transduction of Δ hns::kan^R from BW25113 Δ hns::kan^R into the unmarked BW25113 Δ rppH.

Promoter DNA fragments and in vivo gene expression assays

β -Galactosidase assays were done using the protocol of Miller (1972). All assay values are the mean of three independent experiments with a standard deviation $>10\%$ of the mean. Cells were grown aerobically at 37°C to mid-log phase in LB medium.

Proteins, EMSA, and in vitro transcription assays

H-NS and RNAP were prepared as described (Grainger et al. 2008). DNA for EMSA was generated by PCR using the oligonucleotides listed in Supplemental Table S1. DNA fragments were cut with HindIII, end-labeled using (γ -³²P)-ATP, and used at ~ 10 nM concentration. The in vitro transcription assay was done as described by Grainger et al. (2008). DNA templates were generated by PCR with the oligonucleotides listed in Supplemental Table S2. The template ($\sim 16 \mu\text{g mL}^{-1}$) was preincubated with purified H-NS in 20 mM Tris (pH 7.9), 5 mM MgCl₂, 500 μM DTT, 50 mM KCl, 100 $\mu\text{g mL}^{-1}$ BSA, 200 μM ATP, 200 μM GTP, 200 μM CTP, and 10 μM UTP with 5 μCi (α -³²P)-UTP. The reaction was started with 400 nM *E. coli* RNAP. Transcripts were analyzed on a denaturing polyacrylamide gel.

Bioinformatic analysis of promoters

We used our alignment of 554 *E. coli* promoters to determine selection criteria (Singh et al. 2011). Thus, putative -10 elements were chosen if they matched the sequence 5'-TANAAAT-3', 5'-TATnAT-3' or 5'-TATAnT-3'. The GenoList database (<http://genolist.pasteur.fr>) was used to interrogate bacterial genomes and determine the distribution of potential -10 hexamers between genes and noncoding DNA. We used the list of MvaT-bound

regions described by Castang et al. (2008). Lsr2-bound regions in *M. tuberculosis* H37Rv were determined by applying a cutoff of 1.5 to the ChIP-chip data set of Gordon et al. (2010).

ChIP, Chip-seq, and TSS mapping

Targeted ChIP experiments (Fig. 2) were done as described (Singh and Grainger 2013) using *ehxCABD* fragments cloned in pRW50. For ChIP-seq experiments, cultures were inoculated 1:100 in LB with fresh overnight cultures of MG1655 or MG1655 Δ hns::kan^R, and cells were grown at 225 rpm at 30°C to an OD₆₀₀ of 0.5–0.6. Protocols for ChIP-seq, TSS mapping, and analysis of these data are described in Supplemental Appendix S1.

Acknowledgments

We thank Simon Dove for MvaT ChIP-chip data, and Ding Jin for strains. We thank Randy Morse, Keith Derbyshire, Todd Gray, Doug Browning, and our laboratory members for helpful discussions. We thank the Wadsworth Center Bioinformatics Core Facility and Prateek Sharma for support. Our work was funded by a Wellcome Trust Career Development Fellowship (D.C.G.) and a National Institutes of Health Director's New Innovator Award (IDP2OD007188 to J.T.W.). This material is based on work supported by the National Science Foundation Graduate Research Fellowship under grant number DGE-1060277 (D.M.F.).

References

- Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: The Keio collection. *Mol Syst Biol*. doi: 10.1038/msb4100050.
- Bouffartigues E, Buckle M, Badaut C, Travers A, Rimsky S. 2007. H-NS cooperative binding to high-affinity sites in a regulatory element results in transcriptional silencing. *Nat Struct Mol Biol* **14**: 441–448.
- Browning DF, Busby SJ. 2004. The regulation of bacterial transcription initiation. *Nat Rev Microbiol* **21**: 57–65.
- Castang S, McManus HR, Turner KH, Dove SL. 2008. H-NS family members function coordinately in an opportunistic pathogen. *Proc Natl Acad Sci* **105**: 18947–18952.
- Clark MB, Amaral PP, Schlesinger FJ, Dinger ME, Taft RJ, Rinn JL, Ponting CP, Stadler PF, Morris KV, Morillon A, et al. 2011. The reality of pervasive transcription. *PLoS Biol* **9**: e1000625.
- Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci* **97**: 6640–6645.
- Dame RT, Wyman C, Wurm R, Wagner R, Goosen N. 2002. Structural basis for H-NS-mediated trapping of RNA polymerase in the open initiation complex at the *rrnB* P1. *J Biol Chem* **277**: 2146–2150.
- Dame RT, Noom MC, Wuite GJ. 2006. Bacterial chromatin organization by H-NS protein unravelled using dual DNA manipulation. *Nature* **444**: 387–390.
- Dillon SC, Dorman CJ. 2010. Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. *Nat Rev Microbiol* **8**: 185–195.
- Dornenburg JE, Devita AM, Palumbo MJ, Wade JT. 2010. Widespread antisense transcription in *Escherichia coli*. *MBio* **1**: e00024-10.
- Garcia-Vallve S, Guzman E, Montero MA, Romeu A. 2003. HGT-DB: A database of putative horizontally transferred genes in prokaryotic complete genomes. *Nucleic Acids Res* **31**: 187–189.
- Gordon BR, Li Y, Wang L, Sintsova A, van Bakel H, Tian S, Navarre WW, Xia B, Liu J. 2010. Lsr2 is a nucleoid-associated protein that targets AT-rich sequences and virulence genes in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci* **107**: 5154–5159.
- Gowrishankar J, Harinarayanan R. 2004. Why is transcription coupled to translation in bacteria? *Mol Microbiol* **54**: 598–603.
- Grainger DC, Hurd D, Goldberg MD, Busby SJ. 2006. Association of nucleoid proteins with coding and non-coding segments of the *Escherichia coli* genome. *Nucleic Acids Res* **34**: 4642–4652.
- Grainger DC, Goldberg MD, Lee DJ, Busby SJ. 2008. Selective repression by Fis and H-NS at the *Escherichia coli* *dps* promoter. *Mol Microbiol* **68**: 1366–1377.
- Iyoda S, Honda N, Saitoh T, Shimuta K, Terajima J, Watanabe H, Ohnishi M. 2011. Coordinate control of the locus of enterocyte effacement and enterohemolysin genes by multiple common virulence regulators in enterohemorrhagic *Escherichia coli*. *Infect Immun* **79**: 4628–4637.
- Kahramanoglu C, Seshasayee AS, Prieto AI, Ibberson D, Schmidt S, Zimmermann J, Benes V, Fraser GM, Luscombe NM. 2011. Direct and indirect effects of H-NS and Fis on global gene expression control in *Escherichia coli*. *Nucleic Acids Res* **39**: 2073–2091.
- Keseler IM, Mackie A, Peralta-Gil M, Santos-Zavaleta A, Gama-Castro S, Bonavides-Martinez C, Fulcher C, Huerta AM, Kothari A, Krummenacker M, et al. 2013. EcoCyc: Fusing model organism databases with systems biology. *Nucleic Acids Res* **41**: D605–D612.
- Li H, Granat A, Stewart V, Gillespie JR. 2008. RpoS, H-NS, and DsrA influence EHEC hemolysin operon (*ehxCABD*) transcription in *Escherichia coli* O157:H7 strain EDL933. *FEMS Microbiol Lett* **285**: 257–262.
- Lim CJ, Lee SY, Kenney LJ, Yan J. 2012. Nucleoprotein filament formation is the structural basis for bacterial protein H-NS gene silencing. *Sci Rep* **2**: 509.
- Liu Y, Chen H, Kenney LJ, Yan J. 2010. A divalent switch drives H-NS/DNA-binding conformations between stiffening and bridging modes. *Genes Dev* **24**: 339–344.
- Lodge J, Fear J, Busby S, Gunasekaran P, Kamini NR. 1992. Broad host range plasmids carrying the *Escherichia coli* lactose and galactose operons. *FEMS Microbiol Lett* **74**: 271–276.
- Lucchini S, Rowley G, Goldberg MD, Hurd D, Harrison M, Hinton JC. 2006. H-NS mediates the silencing of laterally acquired genes in bacteria. *PLoS Pathog* **28**: e81.
- Miller J. 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Navarre WW, Porwollik S, Wang Y, McClelland M, Rosen H, Libby SJ, Fang FC. 2006. Selective silencing of foreign DNA with low GC content by the H-NS protein in *Salmonella*. *Science* **313**: 236–238.
- Oshima T, Ishikawa S, Kurokawa K, Aiba H, Ogasawara N. 2006. *Escherichia coli* histone-like protein H-NS preferentially binds to horizontally acquired DNA in association with RNA polymerase. *DNA Res* **13**: 141–153.
- Page L, Griffiths L, Cole JA. 1990. Different physiological roles of two independent pathways for nitrite reduction to ammonia by enteric bacteria. *Arch Microbiol* **154**: 349–354.
- Peters JM, Mooney RA, Grass JA, Jessen ED, Tran F, Landick R. 2012. Rho and NusG suppress pervasive antisense transcription in *Escherichia coli*. *Genes Dev* **26**: 2621–2633.
- Potvin E, Sanschagrin F, Levesque RC. 2008. σ Factors in *Pseudomonas aeruginosa*. *FEMS Microbiol Rev* **32**: 38–55.
- Raghavan R, Sloan DB, Ochman H. 2012. Antisense transcription is pervasive but rarely conserved in enteric bacteria. *MBio* **3**: e00156-12.
- Rogers MT, Zimmerman R, Scott ME. 2009. Histone-like nucleoid-structuring protein represses transcription of the *ehx* operon carried by locus of enterocyte effacement-negative Shiga toxin-expressing *Escherichia coli*. *Microb Pathog* **47**: 202–211.
- Sachdeva P, Misra R, Tyagi AK, Singh Y. 2010. The σ factors of *Mycobacterium tuberculosis*: Regulation of the regulators. *FEBS J* **277**: 605–626.
- Salgado H, Peralta-Gil M, Gama-Castro S, Santos-Zavaleta A, Muñiz-Rascado L, García-Sotelo JS, Weiss V, Solano-Lira H, Martínez-Flores I, Medina-Rivera A, et al. 2013. RegulonDB v8.0: Omics data sets, evolutionary conservation, regulatory phrases, cross-validated gold standards and more. *Nucleic Acids Res* **41**: D203–D213.
- Singh SS, Grainger DC. 2013. H-NS can facilitate specific DNA-binding by RNA polymerase in AT-rich gene regulatory regions. *PLoS Genet* **9**: e1003589.
- Singh N, Wade JT. 2014. Identification of regulatory RNA in bacterial genomes by genome-scale mapping of transcription start sites. *Methods Mol Biol* **1103**: 1–10.
- Singh SS, Typas A, Hengge R, Grainger DC. 2011. *Escherichia coli* σ^{70} senses sequence and conformation of the promoter spacer region. *Nucleic Acids Res* **39**: 5109–5118.
- Tutukina MN, Shavkunov KS, Masulis IS, Ozoline ON. 2007. Intragenic promoter-like sites in the genome of *Escherichia coli* discovery and functional implication. *J Bioinform Comput Biol* **5**: 549–560.

Errata

Genes & Development 28: 1351–1362 (2014)

Functional and molecular features of the Id4⁺ germline stem cell population in mouse testes

Frieda Chan, Melissa J. Oatley, Amy V. Kaucher, Qi-En Yang, Charles J. Bieberich, Cooduvalli S. Shashikant, and Jon M. Oatley

In the above-mentioned article, two x-axis labels in Figure 4E are incorrectly positioned: Tubular-Interstitial (+BV) and Tubular-Interstitial (–BV) should be swapped for the correct presentation.

The authors apologize for any confusion this may have caused.

Genes & Development 28: 214–219 (2014)

Widespread suppression of intragenic transcription initiation by H-NS

Shivani S. Singh, Navjot Singh, Richard P. Bonocora, Devon M. Fitzgerald, Joseph T. Wade, and David C. Grainger

For the above-mentioned article, a revised version of Supplemental Appendix S1 (REV_Appendix_S1.docx) has been posted online to include data accession numbers.



Widespread suppression of intragenic transcription initiation by H-NS

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Genes Dev. 2014 28: 214-219 originally published online January 21, 2014
Access the most recent version at doi:[10.1101/gad.234336.113](https://doi.org/10.1101/gad.234336.113)

Supplemental Material <http://genesdev.cshlp.org/content/suppl/2014/01/15/gad.234336.113.DC1.html>

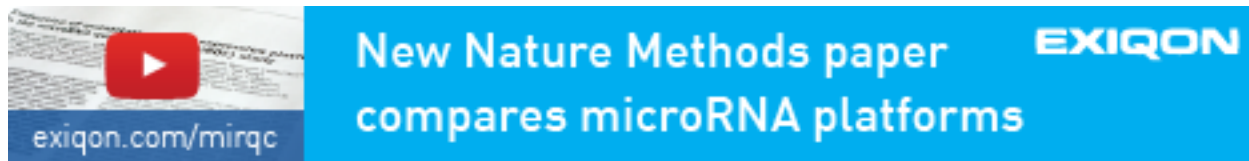
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