The HU Regulon Is Composed of Genes Responding to Anaerobiosis, Acid Stress, High Osmolarity and SOS Induction

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Abstract

Background: The Escherichia coli heterodimeric HU protein is a small DNA-bending protein associated with the bacterial nucleoid. It can introduce negative supercoils into closed circular DNA in the presence of topoisomerase I. Cells lacking HU grow very poorly and display many phenotypes.

Methodology/Principal Findings: We analyzed the transcription profile of every Escherichia coli gene in the absence of one or both HU subunits. This genome-wide in silico transcriptomic approach, performed in parallel with in vivo genetic experimentation, defined the HU regulon. This large regulon, which comprises 8% of the genome, is composed of four biologically relevant gene classes whose regulation responds to anaerobiosis, acid stress, high osmolarity, and SOS induction.

Conclusions/Significance: The regulation a large number of genes encoding enzymes involved in energy metabolism and catabolism pathways by HU explains the highly pleiotropic phenotype of HU-deficient cells. The uniform chromosomal distribution of the many operons regulated by HU strongly suggests that the transcriptional and nucleoid architectural functions of HU constitute two aspects of a unique protein-DNA interaction mechanism.

Introduction

HU is a small, basic, and thermostable dimeric DNA-binding protein initially isolated as a factor stimulating the expression of phage lambda genes [1,2]. It is a major structural component of the nucleoid, and it is conserved among the majority of eubacteria. HU is also present in archaea, in plant chloroplasts, and in a eukaryotic virus [3,4]. HU of E. coli was shown to be a “histone-like protein” which can introduce negative supercoiling into a closed circular DNA in presence of topoisomerase I [5]. We named this protein “HU” where “H” stands for histone and “U” for the U93 strain used at that time to isolate the E. coli nucleoid [1,6]. In most bacteria, HU is encoded by a single gene except in Enterobacteriaceae and Vibrionaceae, which possess two unlinked HU-encoding genes, hupA and hupB [4,7]. In E. coli, single hupA or hupB mutations do not significantly impair growth; however hupA inactivation affects survival in prolonged stationary phase [8,9]. In contrast, the hupAB double mutant grows very slowly and is highly pleiotropic: a number of cell processes, such as cell division, initiation of DNA replication, transcription, and other biochemical functions, are altered and cause a slow-growth phenotype [9,10]. When the absence of HU in E. coli cells is not balanced by compensatory mutations in gyrB, as frequently observed, the hupAB mutant forms very tiny colonies on agar plates [11,12]. It is interesting to note that the HU mutation is lethal in Bacillus subtilis, which has no other histone-like protein [13].

In E. coli, the ratio of the three different HU forms, the HUαβ heterodimer and the HUα2 and HUβ2 homodimers, varies as a function of the growth phase [14]. The three dimers exhibit different DNA binding properties towards particular DNA structures [15] and present different thermodynamic properties [16]. We have shown that HU plays a positive role in translation of the stationary phase sigma factor RpoS [17]. This finding was further substantiated by showing direct HU-RNA interaction [18]. In vitro studies show that HU displays preferential affinity for damaged DNA having nicks or gaps [15,19]. Several reports confirm the involvement of HU in DNA repair: (i) cells lacking HU are extremely sensitive to γ and UV irradiation [20,21]; (ii) HU is capable of displacing LexA, the repressor of the SOS response genes, from its binding sites [22] and (iii) HU binds specifically to a wide array of repair or recombination intermediates [23].

A transcriptional role of HU was also demonstrated for the up-regulation of the proJWX operon in hyperosmolar environments [24], and we showed that HU stimulates transcription by T7 RNA
polymerase [25]. More recently, Adhya’s group revealed a role of HU and negative supercoiling in the formation of the Gal repressor, a nucleoprotein complex necessary to repress transcription of the gal operon [26]. Whereas the respective regulons of other bacterial histone-like proteins, such as Lrp [27], H-NS [28,29], Fis [30], Crp [31], IHF [32] have been identified, the role of E. coli HU on gene regulation has never been addressed systematically at the genomic scale. In the present study, we used microarray hybridization to investigate the pleiotropic role of HU in the cell by studying genome-wide expression as a function of the genetic backgrounds and wild-type backgrounds at three different growth phases. The microarray data, combined with in vivo experiments presented here, confirmed the involvement of HU in the SOS and the osmolarity/supercoiling responses [20,21,24,33]. In addition, the results of these experiments revealed a novel function for this global regulator in the environmental programming of the cellular response during aerobic and acid stress. The interconnection between these various responses and the supercoiled state of the DNA is discussed.

Results

Microarray experiments

E. coli strain C600, originating from the Pasteur Institute, was used for the microarray and in vivo experiments described here (JO2057, Table 1). It was preferred over the commonly used ‘wild type’ strain MG1655 for several reasons: first, most of the genetic and biochemical evidence gathered in our laboratory is based on C600 and second, it has been reported that MG1655 suffers a number of growth defects [34] or chromosomal deletions [35]. Due to the instability of hupA mutants [9,12], special care was taken to reconstruct new mutants starting from JO2057. Strains JO2081 (hupA), JO2087 (hupB) and JO3020 (hupAB) were constructed, and their phenotypes and genotypes were verified, as described in Materials and Methods.

To identify genes regulated by HU, which is present in E. coli as three dimeric forms (HUzβ, HUα2 and HUβ2) at a ratio that varies according to growth phase [14], four strains (the three mutants and the wild type) were grown in LB medium at 37°C. Culture samples for microarray experiments were collected at exponential, transition, and stationary phases. In order to achieve optimal representation of short-lived RNA species, total RNA was extracted from these samples as described in [36]. The genome-wide mRNA levels were measured using high-density E. coli Affymetrix® GeneChips microarrays. A total of 16 microarrays were used: 8 assays were performed to duplicate the data for the wild-type and hupAB double-mutant strains at exponential and stationary phases; the remaining 8 assays consisted of wild-type and hupAB experiments at the transition phase and single hupA and hupB mutants at the three growth phases. The quality of the microarray data was assessed by statistical analysis of the internal duplicated data, which were found, in each case, to be highly reproducible. After Affymetrix MAS 5.0 processing and normalization, a discriminant criterion derived from fold filters used for gene selection [37] was used to identify genes whose expression varied across the experimental conditions. At that stage, 728 out of the 4360 genes composing the microarray (16% of the genome) were retained. This large amount of genes was certainly due to the combined effects of hup genetic background and growth phase. To overcome this difficulty and to identify the genes solely regulated by HU, unsupervised data clustering was performed.

Biological and statistical validation of the E. coli regulon by unsupervised data clustering

Data clustering methods are commonly used to investigate microarray data. However, the relevance of the results is often limited: the number of clusters is not known a priori and has to be specified by the user. To identify meaningful classes of genes regulated exclusively by HU, we developed an unsupervised data-clustering method able to avoid numerous single-gene hypotheses by partitioning the transcriptome profiling data into an optimal and biologically relevant number of clusters and by removing the interference of the unwanted growth phase variable.

We used the K-means algorithm with a distance measure based on the Pearson correlation to cluster the expression profile of each E. coli gene. In our experiments, these profiles were characterized

| Strain, phage, or plasmid | Relevant characteristic(s) or genotype | Source or Reference |
|--------------------------|--------------------------------------|--------------------|
| pRS415                   | lacZ' lacY' bla'                     | [87]               |
| pRS45                    | °. imm21 ind° bla°-lacZ° lacY°        | [87]               |
| pRS88                    | °. imm434 ind° bla°-lacZ° lacY°       | [87]               |
| OHP109                   | hupA:Cm                              | [9]                |
| OHP96                    | hupB:Km                              | [9]                |
| E88                      | ΔftrC::Tn10 (Tc)                      | Jeff Cole          |
| JR1713                   | ΔrecA::Tn10 (Tc)                      | [20]               |
| EN305                    | lacZ::Tn10 (Tc)                       | [25]               |
| JO2057 (C600)            | thr-1 leuB6 thi-1 lacY1 glnV44        | Institut Pasteur, laboratory collection |
| JO2081                   | hupA:Cm (JO2057+P1 transduction from OHP109) | This work         |
| JO2083                   | hupB:Km (JO2057+P1 transduction from OHP96) | This work         |
| JO3020                   | hupA:Cm, hupB:Km (JO2081+P1 transduction from OHP96) | This work         |
| JO2039                   | lacZ::Tn10 (Tc) (JO2057+P1 transduction from EN305) | This work         |
| JO3027                   | lacZ (JO2039 cured from Tn10 with fusaric acid) | This work         |
| JO3029                   | ΔftrC::Tn10 (Tc) (JO2057+P1 transduction from E88) | This work         |
| JO3019                   | ΔrecA::Tn10 (Tc) (JO2057+P1 transduction from JR1713) | This work         |

Table 1. Strains and plasmids used in this work.

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by 12 conditions (4 genotypes at 3 growth phases). The clustering analysis was repeated 24 times for a total number of clusters ranging between 2 and 25. The criterion of Hartigan [38] showed that using nine gene clusters produced the best fit for our data (Fig. 1). We then eliminated unwanted clusters containing genes whose expression varied independently of hup genotypes. For this purpose, the Kruskall-Wallis non parametric tests were used. They permitted us to exclude the following clusters: cluster 1, 3 and 8 (growth phase regulated) and cluster 9 (regulated by an undetermined factor) [Supplemental Table S1]. It was interesting to note that cluster 8 was populated by a number of genes belonging to the stationary phase sigma factor (RpoS) regulon [39] (Supplemental Table S2). Since we have shown previously that RpoS translation is regulated by HU [17], we decided to exclude from our analysis genes characterized as being under RpoS control.

The five selected clusters (Clusters 2, 4, 5, 6 and 7) amounted to 353 genes (8% of the genome) whose transcription varied in the absence of one or both hup genes. These 353 genes constituted the HU regulon. The complete gene list is available in the Supplemental Table S3. With the aid of the RegulonDB Web service [40], these 353 genes were found to correspond to 229 operons [Supplemental Table S4]. Each of the clusters contained a number of complete transcription units; this certainly constituted a good indication that the clustering analysis was consistent with coordinated expression of the individual genes composing operons. The proUVW operon constituted the only exception and will be discussed later. Using the same web resource, each operon of the HU regulon was inspected manually for its respective regulatory characteristic and its potential assignment to other regulons. By comparing these characteristics with our transcriptomic expression patterns, we were able to assign a specific biological significance to each of the five clusters.

The five HU-regulated clusters were characterized as follows. Cluster 2 was the only one to contain genes induced in stationary phase in strains expressing exclusively HUα2 or HUβ2 homodimers; the transcription of these genes in the double mutant and wild-type strain was similar. Most of the genes of Cluster 2 belonged to well characterized regulatory classes: i) genes induced by acid stress, ii) genes responding to high osmolarity and to supercoiling and iii) genes repressed by FNR. Cluster 4 was found to contain genes activated by HUα2 or HUβ2 only in exponential phase; most were FNR activated. Cluster 5 followed an expression pattern opposite to that of cluster 4: its genes were repressed in exponential phase by HUα2 or HUβ2 and corresponded mostly to FNR-repressed genes. The genes of cluster 6 were repressed by the

Figure 1. Gene dataset divided in nine clusters. The five clusters shown in color (2, 4, 5, 6 and 7) constitute the HU regulon. The twelve experimental conditions are represented on the x-axis and correspond to the four genotypes (the wild type JO2057; the hupB JO2083; the hupA JO2081 and the hupAB JO3020) at the three growth phases (exponential, transition and stationary). The y-axis indicates the absolute gene expression values for each experimental condition shown in Supplemental Table 2. The black line corresponds to the average values and the grey interval depicts the standard deviation of the bootstrap analysis.

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HUβ heterodimer in the three growth phases analyzed. Several of the genes in this small cluster belonged to the LexA-repressed SOS regulon. In cluster 7, the genes appeared to be activated by HUβ, mainly at the transition phase: they corresponded to genes stimulated by FNR. These clusters are depicted in Figure 1. It should be noted that a number of HU regulated genes encode chaperones or correspond to oxidative stress genes; they are present in the five clusters (Supplemental Table S5). To validate these results, we undertook a more specific analysis and conducted in vivo experiments to assess the biological relevance of the five HU regulated clusters.

**HU represses SOS response genes (Cluster 6)**

The microarray results showed that transcription of cluster 6 genes was strongly repressed by HUβ at all three growth phases (Table 2). A number of these genes: sulA, umuD, recA, recX, dinI and yebG encode functions that repair DNA damage and prevent cell division until damage has been repaired [41]; they share the property of being highly induced after UV irradiation and repressed by LexA, the repressor of the SOS regulon. The involvement of HU in the SOS response has been reported by us and others: the extreme sensitivity of cells lacking HU to γ and UV irradiation implies that HU participates in DNA repair, probably via a RecA-dependent pathway [20,21,33]. In addition, we reported that the unbalanced over-expression of either HU subunit causes transient SOS induction [42].

SulA is the best known SOS gene; its product binds FtsZ to prevent septum formation in order to inhibit cell division [43]. The constitutive expression of sulA in hupAB mutants has been reported [44]. Derepression of sulA in the hupAB genetic background provides an explanation for cell filamentation previously observed [9]. The UmuD protein belongs to an error-prone repair DNA polymerase [45]. DinI and RecX are involved in the positive and negative modulation of RecA filament formation, respectively [46]. RecA, activated by DNA damage, acts as a coprotease assisting LexA repressor autocleavage [30].

Cluster 6 contained, in addition to SOS induced genes, several genes from the cyanogenic c14 lambdoid prophage: xisE (excisionase), ymfJ and ymFL. These results are compatible with RecA-dependent repressor cleavage and subsequent lytic induction of temperate phages of this family; the SOS-mediated induction of xisE and ymfJ has been reported [47]. Finally, it was noted that Cluster 6 lacked SOS genes responding more weakly to LexA inactivation, but it included some genes with unrelated or complex regulation, such as sodA, which encodes a superoxide dismutase (Supplemental Table S3).

In order to confirm the transcriptome data and to analyze in detail the involvement of HU in the SOS response, we constructed single-copy chromosomal sulA-lacZ and dinI-lacZ fusions (see Materials and Methods) and analyzed their in vivo regulation. Strains JO3057 and JO3059, carrying respectively sulA-lacZ and dinI-lacZ fusions, were tested for SOS response by an antibiogram plate assay in the presence of nalidixic acid. When XGal was present in the plate, a characteristic blue halo was produced at the edge of the growth inhibition zone. The blue halo was not formed by the respective recA mutant derivatives JO3111 and JO3081 (data not shown). In accordance with transcriptome data, the production of β-galactosidase by hupAB fusions strains, JO3111 (sulA-lacZ) and JO3113 (dinI-lacZ), was reproducibly induced three- to five-fold relative to the HU+ parental strains, JO3057 and JO3059 respectively (Fig. 2). Similar results have been described for recA-lacZ and umuC-lacZ fusions [33]. In order to investigate the

### Table 2. Cluster 6 genes.

| Gene Blatter Operon | Stationary WT HupA HupB HupAB | Exponential WT HupA HupB HupAB | Transition WT HupA HupB HupAB |
|---------------------|-------------------------------|--------------------------------|-------------------------------|
| sulA                | 1 1.98 1.09                   | 1 1.69 1.25                   | 1 1.86 0.96                   |
| b1061               | 1 1.09 0.72                   | 1 1.52 1.07                   | 1 1.24 1.04                   |
| b1144               | 1 2.45 0.74                   | 1 1.41 1.41                   | 1 1.23 1.03                   |
| ymfM                | 1 2.67 1.48                   | 1 2.41 1.33                   | 1 1.94 1.17                   |
| b1783               | 1 1.47 1.59                   | 1 1.63 1.24                   | 1 1.35 1.09                   |
| yebG                | 1 0.56 0.87                   | 1 1.34 1.29                   | 1 0.84 0.98                   |
| b1848               | 1 0.56 0.87                   | 1 1.34 1.29                   | 1 0.84 0.98                   |
| yebG                | 1 0.56 0.87                   | 1 1.34 1.29                   | 1 0.84 0.98                   |
| b1147               | 1 4.67 2.33                   | 1 8.91 6.32                   | 1 2.11 1.41                   |
| yebG                | 1 0.56 0.87                   | 1 1.34 1.29                   | 1 0.84 0.98                   |
| b1147               | 1 4.67 2.33                   | 1 8.91 6.32                   | 1 2.11 1.41                   |
| yebG                | 1 0.56 0.87                   | 1 1.34 1.29                   | 1 0.84 0.98                   |
| b1147               | 1 4.67 2.33                   | 1 8.91 6.32                   | 1 2.11 1.41                   |
HU regulates osmolarity/superciling genes (Cluster 2)

In this experiment, we observed that the SOS response still occurred in the wild type fusions measured in HU+ (JO3057, JO3059) and HU− strains (JO3111, JO3113). doi:10.1371/journal.pone.0004367.g002

time course of SOS induction, we measured this response as a function of time, up to 75 min after nalidixic acid induction (Fig. 3). In this experiment, we observed that the SOS response still occurred in a double mutant, as already reported [20], but with a noticeable three-fold lower amplitude. The basal level, before nalidixic acid induction (indicated by an arrow), was higher in the double mutant, as observed in the experiment shown in Figure 2. These results demonstrated that HU is required for a full SOS response.

HU regulates acid-stress genes (Cluster 2)

Cluster 2 also included a number of genes identified as acid inducible in the gene databases. E. coli can withstand a pH of 2.5 for several hours. The acid stress response in E. coli and related organisms is quite complex and involves a number of regulatory mechanisms [54]. Three or potentially four acid-resistance systems (ARS) have been reported [55]. The mechanism involved in the genetic regulation of AR2, which has been extensively investigated, requires only three genes and eleven regulatory proteins. The regulon of two of these (GadX- GadW) has been identified and comprises 15 genes: gadAXW, gadBC, ybaST, slp-yhiF, hdeAB-yhiD, yhiM, yhdD and yhiF [54]. The normalized expression values shown in Table 3 indicate that 13 of these 15 genes belong to cluster 2 of the HU regulon. In addition, Table 3 shows a compilation of acid-inducible genes in four genetic backgrounds (nadX mutant and overexpression of transcriptional regulators EvgA, YdeO and GadX) as reviewed by Foster [56]. A very strong correlation was found between GadX-repressed genes and genes induced in the single hupA or hupB mutants in stationary phase. Cluster 2 also contained genes that respond to acid stress but are not regulated by GadX. These included yhiA (NAD(P):quinone oxidoreductase), ybaA (sodium/proton NhaA transporter), cpaA (a potential chaperone), cyaA (cyclic AMP binding protein), ycbA (Na+/H+ antiporter), ahpC, yedF (isocitrate lyase monomer; malate synthase), ygaU (NAD(P)H:quinone oxidoreductase), ygaM, yebV (NAD(P)H:quinone oxidoreductase), yebS, wrbA, ybaU (NAD(P)H:quinone oxidoreductase), yhiM, yhdD and yhiF [54].

HU is a novel aerobic regulator of energy metabolism (Clusters 2, 4, 5 & 7)

The prominent part of the genes characterizing the HU regulon is known to be involved in bacterial energy metabolism. The amount to 45% of the total number of the regulated operons and are found in four different clusters: 2, 4, 5 and 7 indicating that they obey different expression patterns.

In the facultative anaerobe E. coli, the presence of oxygen and other electron acceptors influences the choice of catabolic and anabolic pathways. E. coli prefers to grow using aerobic respiration, but it can achieve anaerobic respiration with nitrate or other electron acceptors when oxygen is absent; fermentation is used as a last resort when no electron acceptor is available. The expression of enzymes involved in energy metabolism is regulated mainly at the transcriptional level. Two separate oxygen sensing/transcriptional regulatory mechanisms are essential for the aerobic/anaerobic switch (for a review see [58]). First, a two-component system, responsible for micro-aerobic metabolism regulation, is composed of a membrane-bound histidine sensor kinase (ArcB) able to phosphorylate a transcriptional regulator (ArcA) [59]. The E. coli ArcB regulon comprises 175 genes involved in a large number of cell processes [60]. FNR is the second transcriptional sensor-regulator protein involved in the control of anaerobic metabolism; it acts either as a transcription repressor or as an activator. The Fe−S−containing FNR protein is capable of oxygen-regulated dimerization and DNA binding [61]. The E. coli FNR regulon was initially investigated by several laboratories but with somewhat divergent
results probably due to different genetic backgrounds and growth conditions [60,62]. More recently, a report based on a more extensive study has re-evaluated the extent of the FNR regulon [63].

The absence of one or both HU subunits deregulated transcription of the vast majority of the genes encoding electron donors/acceptors or involved in fermentation and in aerobic/anaerobic respiration. A direct comparison of data presented in Clusters 2, 4, 5 and 7 to energy metabolism regulons showed a striking resemblance between the HU and FNR regulons. More surprisingly, the directionality of the HU-FNR regulation was well conserved with a very few exceptions: all the genes induced (or repressed) anaerobically by FNR were also induced (or repressed) by HU in the presence of oxygen. Clusters 2 and 5 contained a majority of FNR down-regulated genes whereas clusters 4 and 7 were populated with a majority of FNR up-regulated genes (See Tables 3, 4, 5 and 6 for the relative, normalized gene expression values). Effectively, HU binding to the FNR regulated, ndh promoter has been reported [64].

In order to investigate and compare the in vivo regulatory relationships between HU and the aerobiosis/anaerobiosis system, we constructed single copy lacZ transcriptional fusions to several genes strongly activated or repressed by HU as described above. We chose three HU-activated genes from cluster 4, namely nirB, narG and dcuC, encoding respectively the large subunit of nitrite reductase, the a subunit of nitrate reductase and the anaerobic C4-dicarboxylate transporter. These genes are known to be positively regulated by FNR [63]. In parallel, two HU-repressed genes from cluster 5, lldP and ndk, encoding respectively the L-lactate permease and the nucleoside diphosphate kinase, were selected on the basis of their strong response in the transcriptome analysis. The transcription of the lldPRD operon, as seen in Figure 5, is repressed anaerobically by ArcA-P [65]. The expression of ndk is negatively controlled in anoxic conditions by an unknown mechanism [62]. In good agreement with microarrays experiments, we observed in vivo that heterodimeric HUβ aerobically repressed lldP and ndk and stimulated nirB, narG and dcuC (Fig. 5A). This is what we observed in anoxic conditions: we confirmed lldP and ndk repression and the induction of nirB, narG and dcuC (Fig. 5B). The expression of β-galactosidase by these five gene fusions was then tested in four genetic backgrounds (wild-type, fnr, hupAB and fnr hupAB) and in aerobic or anaerobic conditions (Fig. 6).

Several observations could be made: (i) the regulatory effect of HU was only apparent in oxic conditions and was stronger for genes that are normally repressed in anaerobiosis, such as lldP and ndk; (ii) in aerobic conditions, there was no significant difference between fnr+ and fnr– strains with the exception a two-fold effect for narG and (iii) in anaerobiosis, we did not observe a significant difference between the HU+ and HU– derivatives of the five gene fusion strains: the only measurable effect was due to the presence/absence of FNR, especially for the FNR-regulated genes nirB, narG and dcuC.

These results prompted us to test the growth phenotype of a hupAB strain in the absence of oxygen on complete anoxic medium (see Materials and Methods). Surprisingly, we noted that the very slow growth phenotype caused by the absence of HU in aerobic conditions was not observed anaerobically. In anoxic conditions, the HU-deficient strain lost its very small colony phenotype and displayed a similar growth rate as the wild type control strain (Fig. 7). After a number of verifications, we concluded that HU was not necessary for growth in the absence of oxygen.
| Gene     | Blattner | Operon   | Exponential Transition Stationary Regulation | Function                                                                 |
|----------|----------|----------|---------------------------------------------|---------------------------------------------------------------------------|
|          |          |          | WT hupA hupB hupAB | WT hupA hupB hupAB | WT hupA hupB hupAB |                                                                           |
| nhaA     | b0019    | nhaAR    | 1 1.37 1.55       | 1 1.47 1.63         | 1 2.18 1.52       | h Na+/H antiporter, pH dependent                                          |
| ybaY     | b0453    | ybaY     | 1 0.91 1.43       | 1 1.25 1.98         | 1 1.32 1.98       | a glycopolypeptide/poly saccharide metabolism                            |
| ybaS     | b0485    | ybaST    | 1 0.91 1.67       | 1 1.65 5.04         | 1 2.44 1.73       | c putative glutaminase                                                   |
| ybaT     | b0486    | ybaST    | 1 1.05 1.32       | 1 1.53 3.33         | 1 1.78 1.54       | c putative amino acid/amine transport protein                           |
| ybgS     | b0753    | ybgS     | 1 0.96 0.98       | 1 0.71 0.84         | 1 1.73 1.21       | a putative homeobox protein                                              |
| dps      | b0812    | dps      | 1 1.14 0.56       | 1 1.39 2.57         | 1 1.8 1.08        | a, b global regulator, starvation conditions                             |
| poxB     | b0871    | poxB-1hhaYhT | 1 0.64 0.99    | 1 1.37 1.19         | 1 1.66 1.55       | a, b pyruvate oxidase                                                    |
| ycaC     | b0897    | ycaC     | 1 0.68 0.73       | 1 1.98 1.64         | 1 1.4 1.59        | a hypothetical protein                                                  |
| cbpA     | b1000    | cbpAM    | 1 0.75 0.83       | 1 0.89 1.65         | 1 1.64 1.52       | a curved DNA-binding protein, functions closely related to DnaJ          |
| wrbA     | b1004    | wrbA-yccJ | 1 0.26 1.3       | 1 1.37 2.02         | 1 0.88 1.17       | a trp repressor binding protein                                          |
| grxB     | b1064    | grxB     | 1 0.92 1.43       | 1 1.29 2.06         | 1 1.09 1.2        | a glutaredoxin 2                                                        |
| sra      | b1480    | sra      | 1 0.65 0.92       | 1 1.77 1.17         | 1 1.66 1.2        | a 30S ribosomal subunit protein S22                                      |
| gadC     | b1492    | gadBC    | 1 0.95 1.52       | 1 1.19 5.17         | 1 2.35 1.98       | d, e Gbc GABA APC transporter                                           |
| gadB     | b1493    | gadBC    | 1 0.67 1.3         | 1 1.95 3.04         | 1 2.71 2.08       | d, e glutamate decarboxylase isom t                                          |
| katE     | b1732    | katE     | 1 0.23 1.95       | 1 1.2 1.63          | 1 1.55 1.7        | a, b catalase hydrogen peroxidas HPII(III)                               |
| osmE     | b1739    | osmE     | 1 0.91 0.97       | 1 1.25 3.82         | 1 1.37 1.44       | a activator of ntrL gene                                                |
| yebV     | b1836    | yebV     | 1 0.81 1.17       | 1 1.1 1.35          | 1 2 1.93         | a, h hypothetical protein                                               |
| otsA     | b1896    | otsA     | 1 0.25 1.08       | 1 1.34 1.81         | 1 1.71 1.74       | a trehalose-6-phosphate synthase                                         |
| otsB     | b1897    | otsA     | 1 0.67 1.12       | 1 2.26 2.48         | 1 2.85 2.16       | a, b trehalose-6-phosphate phophatase                                    |
| yodC     | b1957    | yodC     | 1 0.76 0.98       | 1 1.52 2.07         | 1 2.07 2.22       | a hypothetical protein                                                  |
| fbaB     | b2097    | fbaB     | 1 1.23 1.04       | 1 1.67 2.18         | 1 1.27 1.02       | a fructose bisphosphate aldolase monomer                                 |
| elaB     | b2266    | elaB     | 1 0.74 0.82       | 1 1.35 2.1          | 1 1.46 1.34       | a, b hypothetical protein                                               |
| talA     | b2464    | talA     | 1 1.33 1.55       | 1 1.67 2.31         | 1 2.03 1.62       | a, b transaldolase A                                                     |
| tktB     | b2465    | tktB     | 1 1.81 0.79       | 1 1.26 2.02         | 1 1.56 1.51       | a, b transketolase 2 isom yne                                            |
| ygaU     | b2665    | ygaU     | 1 1 1.15          | 1 1.72 2.45         | 1 1.47 1.5        | a, b hypothetical protein                                               |
| ygaM     | b2672    | ygaM     | 1 0.48 1.04       | 1 2.21 2.24         | 1 1.37 1.25       | a hypothetical protein                                                  |
| yqjC     | b3097    | yqjC     | 1 0.71 1.08       | 1 1.37 1.72         | 1 1.55 1.49       | a hypothetical protein                                                  |
| yqjD     | b3098    | yqjC     | 1 0.69 1.05       | 1 1.63 2.13         | 1 1.49 1.4        | a hypothetical protein                                                  |
| yqjE     | b3099    | yqjC     | 1 0.27 1.16       | 1 1.27 1.63         | 1 1.47 1.47       | a, b Hypothetical protein                                               |
| yrbL     | b3207    | yrbL     | 1 1.29 2.99       | 1 2.12 2.62         | 1 1.47 1.46       | a hypothetical protein                                                  |
| yhiM     | b3491    | yhiM     | 1 3.03 1.24       | 1 0.69 1.71         | 1 1.63 2.38       | a conserved inner membrane protein                                       |
| sip      | b3506    | sip      | 1 0.24 0.88       | 1 1.42 0.64         | 1 2.22 2.44       | d, e, g outer membrane protein induced after carbon starvation; starv ation lipoprotein |
| dctR     | b3507    | sip      | 1 0.24 1.16       | 1 0.98 2.34         | 1 2.28 2.39       | d, e, g protein involved in metabolism of C4-dicarboxylates              |
Table 3. cont.

| Gene   | Blattner | Operon   | Exponential | Transition | Stationary | Regulation | Function                      |
|--------|----------|----------|-------------|------------|------------|------------|-------------------------------|
|        |          |          | WT | hupA | hupB | hupAB | WT | hupA | hupB | hupAB | WT | hupA | hupB | hupAB |
| yhiD   | b3508    | yhiD     | 1  | 1.12 | 2.02 | 3.04  | 1  | 1.21 | 5.16 | 1.01  | 1  | 1.68 | 2.72 | 0.61  |
| hdeB   | b3509    | hdeAB    | 1  | 0.31 | 0.9  | 0.81  | 1  | 2.32 | 14.01| 0.18  | 1  | 2.65 | 2.28 | 0.94  |
| hdeA   | b3510    | hdeAB    | 1  | 0.33 | 0.83 | 0.66  | 1  | 1.76 | 9.13 | 0.15  | 1  | 2.81 | 2.07 | 0.96  |
| hdeD   | b3511    | hdeD     | 1  | 0.36 | 0.82 | 1.39  | 1  | 1.47 | 10.3 | 0.52  | 1  | 2.27 | 2.6  | 0.6   |
| gadE   | b3512    | gadE-mdeEF | 1  | 0.05 | 0.52 | 0.48  | 1  | 1.49 | 12.56| 0.26  | 1  | 4.07 | 5.78 | 1.28  |
| gadW   | b3515    | gadW     | 1  | 0.71 | 0.58 | 1.36  | 1  | 0.96 | 1.76 | 0.91  | 1  | 1.79 | 2.45 | 1.34  |
| gadX   | b3516    | gadX     | 1  | 0.83 | 0.6  | 0.98  | 1  | 1.71 | 1.92 | 1.68  | 1  | 3.15 | 4.25 | 1.66  |
| gadA   | b3517    | gadA     | 1  | 0.37 | 0.2  | 2.88  | 1  | 1.05 | 15.86| 2     | 1  | 3.58 | 4.82 | 1.25  |
| aceB   | b4014    | aceBAK   | 1  | 2.97 | 0.58 | 8.51  | 1  | 0.54 | 0.69 | 4.48  | 1  | 1.8  | 1.02 | 1.11  |
| aceA   | b4015    | aceBAK   | 1  | 6.62 | 1.31 | 2.69  | 1  | 0.34 | 0.33 | 1.26  | 1  | 1.44 | 0.94 | 0.99  |
| aceK   | b4016    | aceBAC   | 1  | 1.55 | 1.04 | 1.19  | 1  | 1.89 | 1.3  | 1.31  | 1  | 1.64 | 0.92 | 0.97  |
| ybj    | b4045    | ybj      | 1  | 0.74 | 1.1  | 1.67  | 1  | 1.94 | 1.32 | 1.73  | 1  | 1.52 | 1.06 | 0.47  |
| yfhK   | b4217    | yfhK     | 1  | 0.36 | 0.81 | 0.93  | 1  | 0.62 | 0.66 | 0.36  | 1  | 1.45 | 1.84 | 0.46  |
| osmY   | b4376    | osmY     | 1  | 1.1 | 1.41 | 2.55  | 1  | 0.6 | 0.67 | 1.78  | 1  | 1.93 | 1.54 | 0.68  |
| ycaC   | b0897    | ycaC     | 1  | 1.68 | 1.67 | 0.73  | 1  | 1.98 | 1.64 | 0.75  | 1  | 1.4 | 1.59 | 0.59  |
| cfa    | b1661    | cfa      | 1  | 0.48 | 0.95 | 0.3   | 1  | 1.17 | 2.37 | 0.2   | 1  | 2.19 | 2.61 | 0.66  |
| yjjD   | b4326    | yjjD     | 1  | 0.57 | 1.54 | 2.41  | 1  | 2.03 | 1.35 | 8     | 1  | 7.42 | 6.38 | 1.23  |

Regulation of genes known to be induced by an increase in osmolarity: (a) genes described in [53], (b) genes described in [52], (c) gene belongs to an operon known to be regulated by osmotic stress; genes known to respond to acid stress: (d) EvgA overexpression, (e) YdeO overexpression, (f) GadX overexpression, (g) gadX mutant [56], (h) genes responding to acid stress independently of GadX and genes controlled by FNR: (FR) FNR-repressed and (FA) FNR-activated [63].

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The E. coli HU Regulon

Discussion

Identification of the HU regulon

We examined the effect of HU, one of the most abundant nucleoid-associated proteins in the bacterial cell, on genome-wide transcription. Since HU exists as three dimeric forms in E. coli (HUαβ, HUα2 and HUβ2), depending mainly on the growth phase, the respective role of each dimer was analyzed. We compared the expression pattern of all E. coli genes in the wild-type host and in strains carrying a mutation in one or in both HU-encoding genes: the hupA, hupB and hupAB mutants. In each case, the cultures were sampled at three different growth phases for two reasons: we had shown that the expression of the HU genes is regulated by growth phase [8] and that the expression of the stationary phase sigma factor, RpoS is stimulated by HU at the translational level [17]. An unsupervised statistical clustering analysis allowed to subtract the interference from growth phase and RpoS and to identify the E. coli genes strictly controlled by HU at the transcriptional level. After this correction, the analysis showed that the transcription of a total of 353 genes composing 229 operons is affected by the lack of one or both HU subunits. The accuracy of the data and its processing was well supported by the number of observed polycistronic operons where all the genes are co-regulated (Supplemental Table S4).

The five HU-regulated clusters identified are populated with genes involved in aerobic/anaerobic energy metabolism and to a lesser extent in the SOS response, osmolarity stress response, and acid stress response. We were able to discriminate between three distinct HU regulons: the HUαβ regulon (cluster 6 repressed by HUαβ and cluster 7 induced by HUαβ), the HUα2 or HUαβ regulons (cluster 4 repressed by HUαβ and cluster 5 repressed by HUα2 in exponential phase) and the HUα2 or HUβ2 regulons (cluster 2, repressed in stationary phase only). Four reasons might explain why we did not observe genes regulated exclusively by HUβ2. First, HUβ2 is unable to introduce negative supercoiling in vitro on a relaxed DNA template in the presence of topoisomerase I [5,8]; second, HUβ2 is normally not present in the cell at 37°C as soon as it is synthesized it forms the heterodimer [14]; third, thermodynamic studies have shown that HUβ2 is partially denatured at 37°C [16], and fourth, the synthesis of HUβ2 is preferentially stimulated during cold shock [66]. The HUβ2 regulon might therefore be linked to low temperature environments.

The largest HU regulon clusters (2, 4, 5 and 7) show striking similarities with the FNR regulon: genes activated or repressed by FNR in anoxic conditions were respectively activated or repressed by HU in the presence of oxygen. Clearly, FNR and HU exert their regulatory control independently: i) the microarray data showed that FNR expression is not affected in hup mutants and ii) the microarray experiments were carried out in aerobic conditions in which FNR is expressed under its apoFNR inactive form [67]. HU could therefore be considered as an aerobic modulator of the FNR regulon.

The transcriptome profiling experiments described in this work showed that a second group of genes, namely the SOS response (or LexA regulon) was induced in the absence of both HU subunits. However, the in vivo experiments, presented in Figure 3, showed that SOS induction is much less efficient in a hupAB background, as observed previously [20]. From these observations, it was possible to conclude that HU is necessary for tight repression as well as for full derepression of the SOS regulon genes found in cluster 6. The “flattening” of the SOS response in the absence of HU could be explained by the capacity of this protein to displace the LexA repressor from its DNA-binding sites [22].

A third group of genes, namely those composing the acid stress or GadX regulon, was found to belong to the HU regulon as well. We tested whether the induction of these genes, induced in the single hupA and hupB mutants, would confer acid resistance in vivo. The acid resistance assay indicated that low pH strongly affected the survival of the hupAB mutant and of the hupA mutant to a lower extent. This effect could be explained by the accumulation of protons intra- or extracellularly due to the deregulation of the cyo and cyd operons encoding cytochrome proton pumps. However, the increase in transcription of acid resistance genes in the hupA and hupB single mutants observed in cluster 2 was insufficient to permit low pH adaptation (Fig. 4).

The HU regulon comprised also a fourth group of genes known to be induced by osmotic shock. The involvement of HU in the adaptation of cell growth in hyperosmolar environments is well known [24]. We observed an excellent correlation between cluster 2 of the HU regulon and genes involved in the synthesis of osmoprotectants, which respond strongly to the osmotic response via DNA supercoiling [53]. Since HU is able to constrain DNA supercoiling and the regulation of these genes requires modulation of DNA superhelicity, it seemed worthwhile to analyze the HU regulon under this perspective.

The HU regulon and DNA supercoiling

The relationship of HU with DNA supercoiling has been analyzed in a number of reports. Nucleoid sedimentation experiments have shown that the absence of HU causes a decrease
Table 4. Cluster 4 genes.

| Gene       | Strain | Operon                  | Exponential   | Transition     | Stationary   | Regulation¹ | Function                                                                 |
|------------|--------|-------------------------|---------------|---------------|--------------|-------------|--------------------------------------------------------------------------|
|             |        |                         | WT           | hupA hupB hupAB | WT           | hupA hupB hupAB | WT           | hupA hupB hupAB |                                                                  |
| dcuC       | b0621  | dcuC                    | 1 0.03 1.22   | 0.08          | 1 0.8 0.87 0.15 | 1 1.03 1.04 0.96 | FA, FAec     | transport of dicarboxylates                                             |
| dmsA       | b0894  | dmsABC                  | 1 0.06 1.82   | 0.14          | 1 1.79 0.78 0.14 | 1 1.04 0.61 1.03 | FA, FAec     | anaerobic dimethyl sulfoxide reductase subunit A                         |
| dmsB       | b0895  | dmsABC                  | 1 0.05 1.59   | 0.13          | 1 2.16 2.19 0.3  | 1 1.5 1.48 1.65 | FA, FAec     | anaerobic dimethyl sulfoxide reductase subunit B                         |
| narK       | b1223  | narK                    | 1 0.02 1.08   | 0.16          | 1 3.44 4.07 7.28 | 1 1.68 1.02 1.26 | FA, FAec     | nitrite extrusion protein                                               |
| narG       | b1224  | narGHJ                  | 1 0.02 1.65   | 0.12          | 1 4.86 4.82 3.78 | 1 1.32 1.99 2.3  | FA, FAec     | nitrate reductase 1, alpha subunit                                       |
| narH       | b1225  | narGHJ                  | 1 0.06 1.64   | 0.13          | 1 2.85 3.71 2.09 | 1 1.13 0.77 1.75 | FA, FAec     | nitrate reductase 1, beta subunit                                       |
| narJ       | b1226  | narGHJ                  | 1 0.06 1.31   | 0.13          | 1 1.89 1.68 1.57 | 1 1.14 1.06 1.36 | FA, FAec     | nitrate reductase 1, delta subunit, assembly function                    |
| narI       | b1227  | narGHJ                  | 1 0.09 1.53   | 0.17          | 1 1.84 1.72 1.63 | 1 1.66 1.09 1.27 | FA, FAec     | nitrate reductase 1, cytochrome b(NR), gamma subunit                    |
| adhE       | b1241  | adhE                    | 1 0.12 1.17   | 0.15          | 1 0.88 0.95 0.22 | 1 0.78 0.97 0.4  | FAec         | CoA-linked acetaldehyde dehydrogenase and iron-dependent alcohol dehydrogenase pyruvate-formate-lyase deactivase |
| napB       | b2203  | napDAGHBC-ccmABCDEFGH    | 1 0.23 2.15   | 0.24          | 1 10.94 8.68 25.5 | 1 2.95 1.93 1.67 | FA, FAec     | cytochrome c-type protein                                              |
| napD       | b2207  | napDAGHBC-ccmABCDEFGH    | 1 0.09 0.72   | 0.22          | 1 1.86 1.76 6.71 | 1 0.84 0.72 0.88 | FA, FAec     | hypothetical protein                                                    |
| napF       | b2208  | napDAGHBC-ccmABCDEFGH    | 1 0.04 0.66   | 0.22          | 1 1.43 1.56 7.67 | 1 1 2.55 2.21  | FA, FAec     | ferredoxin-type protein: electron transfer                              |
| nirD       | b3366  | nirBC-cysG               | 1 0 1.54 0.05 | 1 1.35 0.39 0.72 | 1 2.34 3.43 1.59 | FA            | nitrite reductase (NAD(PH)) subunit                                    |
| nirC       | b3367  | nirBC-cysG               | 1 0.02 1.91   | 0.09          | 1 1.17 1.05 1.79 | 1 1.31 1.15 0.8  | FA            | nitrite reductase activity                                              |
| feoB       | b3409  | feoAB                   | 1 0.12 0.67   | 0.77          | 1 0.16 0.41 0.69 | 1 0.76 1.13 0.93 | FA, FAec     | ferrous iron transport protein B                                        |
| cspA       | b3556  | cspA                    | 1 0.22 1.03   | 0.73          | 1 0.85 0.9 0.6  | 1 0.5 0.39 1.27 | FR           | cold shock protein 7.4; transcripational activator of hns               |
| nifA       | b4070  | nifABCDEFG                | 1 0.14 1.66   | 0.16          | 1 2.25 0.82 1.41 | 1 1.54 1.21 1.16 | FA, FAec     | periplasmic cytochrome c(SS2): plays a role in nitrite reduction         |
| nifB       | b4071  | nifABCDEFG                | 1 0.09 2.16   | 0.07          | 1 4.15 1.19 1.51 | 1 4.71 3.62 2.6  | FA, FAec     | formate-dependent nitrite reductase a penta-haeme cytochrome c           |
| nifC       | b4072  | nifABCDEFG                | 1 0.14 2.19   | 0.15          | 1 2.8 1.3 0.84  | 1 1.76 1.62 0.69 | FA, FAec     | formate-dependent nitrite reductase Fe-5 centers                        |
| yjJ/K      | b4128  | yjJ/K                   | 1 0.03 1.63   | 0.32          | 1 1.06 1.65 0.92 | 1 1.82 1.15 0.81 | FA            | hypothetical protein                                                    |
| yjJ/W      | b4379  | yjJ/W                   | 1 0.05 1.51   | 0.31          | 1 2.21 1.6 0.11  | 4.19 0.57 0.53 | FA            | putative activating enzyme                                              |
| yjJ/L      | b4380  | yjJ/L                   | 1 0.03 1.39   | 0.27          | 1 1.88 1.5 0.31  | 1 1.58 1.45 0.94 | FA            | hypothetical protein                                                    |

¹Regulation symbols: FA: FNR-activated and FR: FNR-repressed [63]; FAec: FNR-activated (Ecocyc: http://ecocyc.org).

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### Table 5. Cluster 5 genes.

| Gene | Blattner | Operon | Exponential WT | Transition WT | Stationary WT | Regulation | Function |
|------|----------|--------|----------------|---------------|---------------|------------|----------|
|      |          |        | hupA hupB hupAB| hupA hupB hupAB| hupA hupB hupAB|            |          |
| cyoD | b0429    | cyoABCDE| 1 2.14 0.73 1.63| 1 0.03 0.04 0.73| 1 0.34 0.59 0.58| FA, FRec  | cytochrome o ubiquinol oxidase subunit IV |
| cyoC | b0430    | cyoABCDE| 1 2.23 0.81 1.56| 1 0.07 0.06 0.75| 1 0.51 0.53 0.64| FA, FRec  | cytochrome o ubiquinol oxidase subunit III |
| cyoB | b0431    | cyoABCDE| 1 2.25 0.84 1.67| 1 0.07 0.06 1.02| 1 0.43 0.43 0.73| FA, FRec  | cytochrome o ubiquinol oxidase subunit I  |
| cyoA | b0432    | cyoABCDE| 1 2.71 0.84 2.37| 1 0.18 0.1 1.52| 1 0.47 0.53 0.75| FA, FRec  | cytochrome o ubiquinol oxidase subunit II |
|      |          |        |                |               |               |            |          |
| sdcC | b0721    | sdcDAB-b0725-sucABCD| 1 13.94 0.99 7.68| 1 0.29 0.17 1.57| 1 0.36 1.01 1.02| FRec      | fumarase A = fumarate hydratase Class I aerobic isozyme |
| sdcD | b0722    | sdcDAB-b0725-sucABCD| 1 9.23 0.79 5.2| 1 0.23 0.15 1.7| 1 0.42 0.71 0.79| FA         | putative periplasmic binding transport protein |
| sdcA | b0723    | sdcDAB-b0725-sucABCD| 1 7.2 0.82 4.21| 1 0.15 0.16 1.08| 1 0.29 0.55 0.63| FRec      | succinate dehydrogenase, flavoprotein subunit |
| sdcB | b0724    | sdcDAB-b0725-sucABCD| 1 7.53 0.76 3.67| 1 0.14 0.16 1.08| 1 0.32 0.63 0.53| FRec      | succinate dehydrogenase, iron sulfur protein |
| bo725 | b0725 | sdcDAB-b0725-sucABCD| 1 4.65 0.66 2.64| 1 0.18 0.22 0.72| 1 0.38 0.88 0.49| FRec      | putative periplasmic binding transport protein |
| sucA | b0726    | sdcDAB-b0725-sucABCD| 1 2.71 0.7 2.28| 1 0.15 0.22 0.74| 1 0.26 0.56 0.45| FRec      | succinate dehydrogenase (decarboxylase component) |
| sucB | b0727    | sdcDAB-b0725-sucABCD| 1 1.83 0.67 1.79| 1 0.15 0.2 0.7| 1 0.29 0.6 0.62| FRec      | succinate dehydrogenase (dihydrolipoamide transsuccinylase E2 component) |
| sucC | b0728    | sdcDAB-b0725-sucABCD| 1 1.81 0.63 1.97| 1 0.16 0.19 0.84| 1 0.33 0.72 0.66| FRec      | succinyl-CoA synthetase, beta subunit |
| sucD | b0729    | sdcDAB-b0725-sucABCD| 1 2.26 0.69 1.95| 1 0.14 0.14 0.62| 1 0.42 0.74 0.49| FRec      | succinyl-CoA synthetase, alpha subunit |
| fumA | b1612    | fumA   | 1 5.47 0.82 3.05| 1 0.34 0.23 1.13| 1 0.49 0.47 1.55| FRec      | fumarase A = fumarate hydratase Class I aerobic isozyme |
| fliY | b1920    | fliAZY | 1 1.85 0.69 1.85| 1 1.11 0.91 3.26| 1 1.42 1.01 0.73| FA         | putative periplasmic binding transport protein |
| ndk | b2518    | ndk    | 1 16.55 0.94 12.98| 1 0.67 0.3 8.27| 1 0.8 0.98 5.24| This work| nucleoside diphosphate kinase |
| ldp | b3603    | ldpPRD| 1 49.62 0.9 17.04| 1 0.86 0.54 2.38| 1 0.58 0.53 0.65| This work| L-lactate permease |
| ldpR | b3604    | ldpPRD| 1 29.89 0.79 15.07| 1 0.07 0.13 0.33| 1 0.73 0.54 0.86| This work| transcriptional regulator |
| ldfA | b3605    | ldpPRD| 1 12.6 0.79 7.63| 1 0.21 0.15 0.56| 1 0.68 0.58 0.82| This work| L-lactate dehydrogenase |
| fimA | b4314    | fimABCDFGH| 1 0.34 0.81 2.8| 1 0.22 0.63 2.56| 1 0.49 0.43 3.68| FA         | major type 1 subunit fimbrin (pilin) |
| fimI | b4315    | fimABCDFGH| 1 0.45 1.28 5.45| 1 0.58 0.88 5.27| 1 1.28 1.29 5.09| FA         | fimbrial protein |
| fimC | b4316    | fimABCDFGH| 1 0.5 1.09 6.36| 1 0.74 1.04 8.35| 1 1.32 1.17 3.75| FA         | periplasmic chaperone, required for type 1 fimbriae |

1Regulation symbols: FA: FNR-activated and FR: FNR-repressed [63]; FRec: FNR-repressed (Ecocyc: http://ecocyc.org).

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### Table 6. Cluster 7 genes.

| Gene | Blattner Operon | Exponential Transition Stationary | Regulation | Function |
|------|----------------|----------------------------------|------------|----------|
|      | WT | hupA | hupB | hupAB | WT | hupA | hupB | hupAB | WT | hupA | hupB | hupAB |
| aceE | b0114 | pdrR-aceEF-lpd | 1 | 0.44 | 1.16 | 0.71 | 1 | 1.37 | 1.51 | 0.35 | 1 | 0.78 | 0.75 | 1.02 | FR, FRec | pyruvate dehydrogenase (decarboxylase component) |
| aceF | b0115 | pdrR-aceEF-lpd | 1 | 0.46 | 1.35 | 0.65 | 1 | 1.51 | 1.58 | 0.41 | 1 | 0.74 | 0.73 | 1.04 | FR, FRec | pyruvate dehydrogenase (dihydrolipoyltransacetylase component) |
| ycbU | b0191 | ycbU | 1 | 0.05 | 1.23 | 0.16 | 1 | 0.72 | 0.96 | 0.37 | 1 | 0.89 | 0.93 | 0.7 | FA | hypothetical protein |
| ndh | b1109 | ndh | 1 | 0.05 | 0.81 | 0.54 | 1 | 0.85 | 1.9 | 0.34 | 1 | 1.44 | 0.88 | 0.32 | FR, FRec | respiratory NADH dehydrogenase |
| ompW | b1256 | ompW | 1 | 0.19 | 1.32 | 0.29 | 1 | 1.66 | 1.42 | 0.34 | 1 | 0.43 | 0.41 | 2.29 | FA, FAc | putative outer membrane protein |
| fdnG | b1474 | fdnGHI | 1 | 0.12 | 1.39 | 0.36 | 1 | 6.69 | 3.57 | 2.55 | 1 | 1.06 | 0.91 | 0.98 | FA, FAc | formate dehydrogenase-N, nitrate-inducible, alpha subunit |
| ydfZ | b1541 | ydfZ | 1 | 0.17 | 0.96 | 0.39 | 1 | 3.97 | 3.66 | 1.14 | 1 | 1.22 | 1.23 | 1.39 | FA | hypothetical protein |
| ydhY | b1674 | ydhYVW | 1 | 0.3 | 1.34 | 0.74 | 1 | 0.77 | 1.99 | 0.36 | 1 | 1.96 | 0.88 | 1.37 | FA | putative outer membrane protein, Fe-S subunit |
| yesU | b1800 | yesU | 1 | 0.34 | 0.41 | 2.67 | 1 | 0.15 | 0.44 | 0.02 | 1 | 0.62 | 0.9 | 0.3 | FR | putative tartrate dehydrogenase |
| glpA | b2241 | glpABC | 1 | 0.4 | 1.24 | 0.28 | 1 | 1.02 | 0.89 | 0.35 | 1 | 0.35 | 0.36 | 0.55 | FA, FAc | sn-glycerol-3-phosphate dehydrogenase (anaerobic), large subunit |
| glpB | b2242 | glpABC | 1 | 0.44 | 1.12 | 0.23 | 1 | 1.16 | 0.96 | 0.24 | 1 | 0.28 | 0.29 | 0.54 | FA, FAc | sn-glycerol-3-phosphate dehydrogenase (anaerobic), membrane anchor subunit |
| glpC | b2243 | glpABC | 1 | 0.54 | 1.24 | 0.31 | 1 | 1.2 | 1.17 | 0.27 | 1 | 0.26 | 0.33 | 0.51 | FA, FAc | sn-glycerol-3-phosphate dehydrogenase (anaerobic), K-small subunit |
| uraA | b2497 | upp-uraA | 1 | 0.42 | 1.03 | 0.49 | 1 | 0.66 | 0.07 | 0.8 | 1 | 0.93 | 1.11 | 0.95 | FA, FAc | uracil transport |
| upp | b2498 | upp-uraA | 1 | 0.37 | 0.83 | 0.53 | 1 | 0.76 | 0.39 | 0.79 | 1 | 0.85 | 0.91 | 1.18 | FA, FAc | uracil phosphoribosyltransferase |
| yflD | b2579 | yflD | 1 | 0.04 | 0.95 | 0.41 | 1 | 2.29 | 2.13 | 0.54 | 1 | 0.4 | 0.31 | 0.98 | FA, FAc | putative formate acetyltransferase |
| gcvH | b2904 | goTHP | 1 | 0.92 | 0.96 | 0.55 | 1 | 1.08 | 1.01 | 0.59 | 1 | 0.21 | 0.38 | 0.24 | FA, FAc | in glycin cleavage complex, carrier of amino-methyl moiety via covalently bound lipoyl cofactor |
| ansB | b2957 | ansB | 1 | 0.01 | 1.55 | 0.18 | 1 | 2.87 | 1.38 | 0.23 | 1 | 0.7 | 1.3 | 1.51 | FA, FAc | periplasmic L-asparaginase II |
| uxaA | b3091 | uxaCA | 1 | 2.56 | 1.01 | 0.63 | 1 | 1.46 | 0.56 | 0.11 | 1 | 0.51 | 0.47 | 0.6 | FA, FAc | altronate hydrolase |
| uxaC | b3092 | uxaCA | 1 | 1.17 | 0.84 | 0.59 | 1 | 2.31 | 0.64 | 0.11 | 1 | 0.78 | 0.67 | 1.06 | FA, FAc | uronate isomerase |
| tdcF | b3113 | tdcABCDEF | 1 | 0.87 | 1.15 | 0.44 | 1 | 16.22 | 14.14 | 0.51 | 1 | 1.29 | 1.12 | 1.21 | FA, FAc | hypothetical protein |
| tdcE | b3114 | tdcABCDEF | 1 | 0.68 | 1.11 | 0.29 | 1 | 18.25 | 15.59 | 0.35 | 1 | 1.29 | 1.27 | 1.25 | FA, FAc | probable formate acetyltransferase 3 |
| tdcD | b3115 | tdcABCDEF | 1 | 0.51 | 1.94 | 0.33 | 1 | 4.3 | 3.86 | 0.08 | 1 | 1.38 | 1.14 | 1.2 | FA, FAc | putative kinase |
| tdcC | b3116 | tdcABCDEF | 1 | 0.35 | 1.67 | 0.25 | 1 | 2.3 | 2.12 | 0.04 | 1 | 0.8 | 0.76 | 0.77 | FA, FAc | anaerobically inducible L-threonine, L-serine permease |
Table 6. cont.

| Gene    | Blattner Operon | Stationary | Transition | Exponential |
|---------|-----------------|------------|------------|-------------|
| hupA    |                  |            |            |             |
| hupB    |                  |            |            |             |
| hupAB   |                  |            |            |             |
| b3117   |                  |            |            |             |
| tdcB    | tdcABCDEFG      |            |            |             |
|        | FA, FAec        |            |            |             |
|        | threonine       |            |            |             |
|        | dehydratase     |            |            |             |
|        | catabolic       |            |            |             |
| b3118   |                  |            |            |             |
| tdcA    | tdcABCDEFG      |            |            |             |
|        | FA, FAec        |            |            |             |
|        | transcriptional  |            |            |             |
|        | activator of    |            |            |             |
|        | tdc operon      |            |            |             |
| malP    |                  |            |            |             |
| b3417   | malPQ           |            |            |             |
|        | FA, FAec        |            |            |             |
|        | maltodextrin    |            |            |             |
|        | phosphorylase   |            |            |             |
| b3942   | katG            |            |            |             |
|        | katG            |            |            |             |
|        | FA, FAec catalase|            |            |             |
|        | hydroperoxidase HPI(I) | | | |
| b4122   | fumB            |            |            |             |
|        | dcuB-fumB       |            |            |             |
|        | FA, FAec        |            |            |             |
|        | fumarase        |            |            |             |
|        | fumarate        |            |            |             |
|        | hydratase Class |            |            |             |
|        | I anaerobic     |            |            |             |
|        | isozyme         |            |            |             |
| b4123   |                  |            |            |             |
| tdcG    | frdABCD         |            |            |             |
|        | frdABCD         |            |            |             |
|        | frdA            |            |            |             |
|        | frdB            |            |            |             |
|        | frdC            |            |            |             |
|        | frdD            |            |            |             |
|        | fumarate        |            |            |             |
|        | reductase       |            |            |             |
|        | anaerobic       |            |            |             |
|        | membrane        |            |            |             |
|        | anchor polypeptide |            |            |             |
|        | subunit         |            |            |             |
| b4471   |                  |            |            |             |
|        | tdgA            |            |            |             |
|        | L-serine        |            |            |             |
|        | deaminase 3     |            |            |             |

Regulation symbols: FA: FNR-activated and FR: FNR-repressed [63]; FRec: FNR-repressed and FAec: FNR-activated (Ecocyc: http://ecocyc.org).

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To further investigate the link between HU and DNA superhelicity, we performed a systematic comparison between the HU regulon and the lists of genes under supercoiling control established independently by two groups [72,73]. We observed that the HU regulon shared very few genes (<8% and <4%, respectively) with the superhelicity regulons (Supplemental Fig. S1 A & Tables S6 to S9). This shared subset of the HU regulon contained genes regulated by supercoiling and osmolarity (otsB) or by supercoiling and acid stress (nhaA and gadB). The same comparison was repeated with the regulons of the two other major nucleoid proteins H-NS and Fis [72] with a very similar outcome (Supplemental Fig. S1 B, C & Tables S10 to S14). We deduced from these observations that the majority of genes under transcriptional superhelical control are regulated by unconstrained chromosomal supercoiling and not by the constraining activity of HU, H-NS and Fis. The regulons of these three proteins were then compared to analyze their respective contribution to global regulation.

Global regulation by HU, H-NS and Fis

Identification of the HU regulon permitted the systematic comparison with the respective regulons described recently of the other major nucleoid-associated proteins H-NS and Fis [72]. Taken together, these three abundant proteins are responsible for most of the compaction of the bacterial chromosome: it has been reported that half of the negative supercoiling is constrained by Fis, H-NS and HU [74]. Our data indicated that HU, H-NS and Fis regulons share 15% to 32% of their genes, while specific genes range from 59% to 69%; only 26 genes are common to the three regulons (Supplemental Fig. S1 D & Tables S15 to S18). Several genes are co-regulated by HU/Fis and H-NS and encode proteins that repress the acid stress response genes and the biosynthesis of fimbriae, whereas both induce flagellar biosynthetic genes (Supplemental Table S15). A number of chaperone genes and environmental stress response genes are differentially regulated by HU and H-NS (Supplemental Table S15). By comparing the genes co-regulated by HU and Fis, we observed that both proteins induce Cluster 4 genes while they repress Cluster 5 genes (Supplemental Table S16). We observed also that HU regulates these two clusters in the exponential phase, at the stage of growth where Fis is most actively synthesized [14].

HU-DNA binding and transcription regulation

The identification of a regulon assumes that its regulator interacts with specific genes, upstream of the protein coding sequence. How does HU recognize its targets? The overlap between the FNR and HU regulons suggested that FNR binding-site variants might be recognized by HU. We therefore investigated, by Gibbs sampling, the promoter region of the regulated operons in each cluster. This search failed to produce significant shared sequence motif (data not shown). It is interesting to note that FNR can bind to some of its targets in the absence of a
canonical FNR binding sequence, suggesting cooperative binding with another factor [75].

Among the various HU-nucleic acid binding properties that have been described, different DNA binding modes can be invoked to explain mechanistically its regulatory function. Namely, HU contributes to DNA loop formation [26], is capable of constraining supercoiling DNA [69], and shows higher affinity for distorted DNA structures [23].

The HU regulon is composed of four well defined biological classes of genes involved in stress response and adaptation to environmental shifts. These four classes can be divided into two categories on the basis of the reported DNA binding modes of HU, described above. The regulation of the genes in first category requires, in addition to HU, specific DNA binding of the regulatory proteins LexA, GadX or FNR. We hypothesize that HU induces DNA looping to help loading/unloading of these regulators onto their specific binding sites in order to allow/block RNA polymerase transcription initiation. The formation of such a complex, called “repressosome,” has been studied in detail for the gal operon. It involves the participation of the GalR repressor, HU,

Figure 5. Regulation of cluster 4 and 5 genes by HU and aerobiosis. (A) Comparison of the beta-galactosidase activity of lidp, ndk, nirB, narG and dcuC transcriptional lacZ fusions in wild type and hupAB strains. (B) Comparison of the beta-galactosidase activity of the same gene fusions in aerobic and anaerobic conditions. The numbers under the bars correspond to strain numbers described in Table 7.

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and negatively supercoiled DNA [26]. A similar phenomenon has been reported for the control of the FNR-regulated ndk gene [64].

The second category of the HU regulon contains genes known to be controlled primarily by DNA supercoiling during hyperosmolar upshift without the involvement of a specific regulatory protein [53]. For these genes, it appears that the driving force of regulatory control is solely constituted by the superhelical DNA constraining capability of HU; effectively, these genes are not found in the reported supercoiling regulons [72, 73].

We also observed a good correlation between genes regulated by HU and the chromosomal regions exhibiting “extreme structure” predicted by the group of Ussery: these authors analyzed five parameters affecting the DNA conformation of the E. coli chromosome and identified 36 sites presenting a maximal distortion [76]. We found that 15 of these sites mapped in (or very near) genes belonging to the HU regulon (Supplemental Table S19). This observation is consistent with the preferred interaction of HU with distorted DNA structures rather than sequences [15, 19].

Global regulatory function and structural role of HU

How could we reconcile the transcriptional and chromosomal architectural roles of HU? We have shown here that HU controls the transcription of 353 genes composing 229 operons. Phenotypically, E. coli hupAB strains grow very poorly and display numerous nucleated cells. These deleterious effects might be caused by the inverted expression pattern of stress-induced genes and energy metabolism operons and to loss of the nucleoid architecture. Interestingly, these phenotypes are only visible in the absence of oxygen and are rescued under anoxic conditions. The absence of HU regulatory effect in anaerobiosis can be explained by the increase of negative supercoiling, in these conditions, due to an increase in DNA gyrase activity [68] or a decrease in topoisomerase I activity [77]. As shown by our phenotype observation in Figure 7, normal anoxic growth of hupAB strains suggests that, under these conditions of absence of oxygen, the superhelical DNA constraining activity of HU is not required for the organization of the bacterial nucleoid.

In aerobic conditions, however, the essential role of HU could be illustrated as follows. If we consider the presence of 30,000 HU dimers in the cell [6, 78], each covering a 9 bp sequence [79], it can be deduced that each of the 229 HU-regulated promoters accommodates 130 dimers, binding cooperatively, over a 1200 bp segment. In these conditions, the average spacing between HU binding sites on the chromosome would amount to ∼20 kb (Supplemental Figure S2). Interestingly, the bacterial nucleoid has been described as being shaped in domains of 50–100 kb [80] whereas more recent studies re-evaluated the organization of the chromosome in 400 supercoiled looped domains of ∼10 kb (reviewed in [81]). The formation of these high-order HU-DNA complexes has been observed by techniques as diverse as crystallography [69–71], atomic force microscopy [82] and fluorescence resonance energy transfer [83]. We therefore propose to localize the nucleoid-shaping and DNA-constraining roles of HU at the 229 chromosomal sites where transcription regulation occurs.

In conclusion, our data has shown that HU regulates the expression of 8% of the E. coli genome using two mechanisms. In the first, HU cooperates with known transcription regulators such as LexA, GadX of FNR and in the second, HU acts alone on its DNA structure targets. Our observation that HU is necessary in aerobiosis and dispensable in anoxic conditions unravels the important role played by this histone-like protein in the metabolism of the bacterial cell and opens new areas for research to be explored.

Materials and Methods

Plasmids, phages, bacterial strains and general growth conditions

The E. coli K-12 bacterial strains used in this work are listed in Table 1. New C600 (JO2057) derivatives carrying the mutated hup were constructed. JO2081 (hupA), JO2083 (hupB) and JO3020 (hupAB) were obtained by phage P1 transduction by selecting on LB agar plates containing the appropriate antibiotics. The C600 hupAB mutant displayed the characteristic small-colony and cell-filamentation phenotypes, as expected and observed previously [9]. The hup gene interruptions were verified for each construction by PCR analysis of genomic DNA extracted from each mutant (data not shown). The absence of the respective HU subunits was demonstrated by western blot immunodetection after SDS-PAGE and Acid Urea Triton-PAGE (data not shown). The lacZ::fus and recA mutations, originating respectively from ENS303 [25], EF88

Figure 6. Regulation of cluster 4 and 5 genes by HU, aerobiosis and FNR. Individual and combined effects of the wild type, Δfnr, hupAB and Δfnr hupAB backgrounds and aerobic or anaerobic growth conditions on the beta-galactosidase activity of lldp, ndk, nirB, narG and dcuC transcriptional lacZ fusions. The numbers under the bars correspond to strain numbers described in Table 7.

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Jeff Cole), and JR1713 [20], were introduced into the same genetic background by P1 transduction to generate respectively JO2039, JO3019 and JO3029. Due to the lack of phenotype in our laboratory culture conditions, the presence of the fnr::Tn10 marker in JO3029 was verified by genomic PCR (data not known). To allow re-use of the tetracycline resistance marker, strain JO2039 was cured from its Tn10 transposon by growth on fusaric acid medium to yield JO3027 as described [84]. Luria-Bertani (0.5% NaCl) broth and agar (15 g/liter) were used for routine growth. When used, ampicillin, tetracycline, kanamycin, and chloramphenicol were provided at final concentrations of 100, 12, 50, and 20 μg/ml, respectively. Anoxic growth conditions were achieved in a 2.5 liter Oxoid anaerobic jar (Model AG25) (Oxoid, Hampshire, UK) or in a Coy anaerobic chamber (Coy Enterprises, Inc.) using LB 0.5% NaCl containing 10 mM NaNO₃ and 0.2% (wt/vol) glucose.

Construction of strains carrying single copy promoter-lacZ fusions and mutant derivatives

The lacZ fusion strains used in this work are shown in Table 7. They were constructed as follows. E. coli chromosomal DNA was extracted and purified from strain JO2057, as described [85]. The cloning of the promoters of the genes of interest was assisted by the BAGET web service [86]. They were amplified by PCR from the chromosome with Pfu polymerase (Promega) using gene-specific primers flanked by EcoRI or BamHI restriction sites except in the case of sulA where the second primer was flanked by a BglII site due to the presence of a BamHI site in the amplified fragment. Theses oligonucleotides are described in Supplemental Table S20. The resulting PCR fragments were digested with EcoRI and BamHI (EcoRI and BglII for sulA) and directionally cloned into BamHI-EcoRI-digested lacZ operon fusion vector pRS415 [87]. All lacZ fusions were transferred from their respective plasmid to phage λRS45 by lytic rescue with the exception of the dinI::lacZ and sulA::lacZ fusions which were rescued on the non SOS-inducible λRS88 ind-. C600 lacZ lysogens were constructed with these fusion-carrying phages. Additional mutations were then introduced by P1-mediated transduction and selection for the appropriate antibiotic resistance. In order to avoid gene dosage interference caused by a variable number of fusion-carrying prophages, the strain derivatives were constructed sequentially using the original C600 lacZ lysogen as starting material, as indicated in Table 7.

Beta-galactosidase assay

Cell extracts were prepared from exponential phase cultures grown in 5 ml LB at 37°C, aerobically or anaerobically as described above. Assays of β-galactosidase from these extracts were carried out as described [88], in triplicate.

Acid resistance assay

The assay to measure the resistance of strains to low pH exposure was conducted in duplicate, as described [57].

Microarray technology

Affymetrix GeneChips were chosen for the transcriptomic approach, since they provide a 15- to 40-fold probe redundancy for each individual gene to increase repeatability. In our hands, the correlation between duplicate experiments was statistically significant (see below). The four bacterial strains JO2057, JO2081, JO2083 and JO3020 (described above) were grown in 100 ml LB 0.5% NaCl at 200 rpm in a New Brunswick laboratory shaker in 2-liter flasks. LB medium was chosen over synthetic minimal for its better permissivity for the growth of hupAB mutants. The typical doubling time, observed in exponential phase, was 40 min for JO2057, JO2081, JO2083 and 75 min for JO3020. The various growth phase samples were collected at the following cell densities: exponential phase: OD₆₀₀ 0.6–0.7; transition: 2.2–2.5 and stationary: 4.6–4.8 (3.0 for hupAB).
Special care was taken to process the samples immediately for total RNA extraction to ensure optimal representation of short lived messenger species. The protocol for RNA extraction was adapted form [36]. Briefly, a culture volume of 7 ml was mixed with the same volume of boiling 2% SDS, 4 mM EDTA and heated at 100°C for 5 to 3 min then vortexed cooled first. At this stage, the extract was either processed further or stored at −20°C. Seven milliliter of phenol/water were added before incubating 10 min at 67°C with occasional stirring. The samples were cooled on ice and centrifuged 10 min at 5000 rpm at 4°C. The aqueous phase was separated, extracted the same way and then once with phenol/chloroform (v/v 1:1). One tenth volume of 4 M NaCl and 2.5 volumes of cold ethanol were then added to the aqueous phase. The pellets were left at −20°C for two hrs and then centrifuged at 8500 rpm at 4°C. The pellet was washed with 70% ethanol, dried under vacuum, and resuspended in 300 µl sterile water and transferred to an eppendorf tube. Qiagen RMD buffer (34.5 µl) and of RNase free DNase I (9.37 µl, Qiagen) were added. After 15 min at room temperature, the tubes were mixed by inversion and deproteinized as described above with 300 µl phenol/H₂O at room temperature. The RNA was then precipitated with 37.5 µl NaCl 4 M and 823 µl cold ethanol. After 2 hrs at −20°C, the tubes were centrifuged 30 min at 10,000×g at 4°C, the pellets were then washed with 70% ethanol then dried under vacuum and resuspended in 60 µl sterile water. The RNAs were stored at −20°C. RNA purity was assessed by measuring the A_{260}/A_{280} ratio and selecting them within a range of 1.8 to 2.1. Samples with a ratio lower than of 1.8 were discarded. RNA samples were reverse transcribed and biotinylated according to the Affymetrix protocol. Biotin-labeled cDNA (2.5 µg) was hybridized to E. coli antisense genome arrays (Affymetrix) at 45°C for 16 h as recommended in the GeneChip technical manual (Affymetrix). The probed arrays were scanned at 570 nm using a confocal laser scanner (Hewlett-Packard G2500A). Microarray Suite 3.0 software (Affymetrix) was used to determine the gene expression levels. The Affymetrix Genechips were used for this purpose as follows. The most relevant experiments were carried out in duplicate: the wild type (JO2057) and the hupAB (JO3020) strains were tested in the exponential and stationary phase. The last chips were used to test, respectively, the hupA (JO2081) and single hupB (JO2083) mutants at the three phases.

**Data driven, unsupervised statistical methodology**

Affymetrix microarray hybridization signals were normalized with dChip [89]. Hybridization signals and detection calls in MIAME-compliant format have been deposited in the NCBI GEO database (accession #GSE11183). A total of 4368 annotated genes were further processed. Due to the large number of regulated genes, we used a very restrictive selection criterion as follows: the genes whose expression varied significantly in at least one of the conditions were identified by comparing their maximal (MaxVal) and minimal (MinVal) expression values in each experimental condition with the following criterion:

\[
\log_2(\text{MaxVal} - \text{MinVal}) > 8
\]

derived from fold filters used for genes selection. Genes were selected for further analysis if they presented both relative ([MaxVal/MinVal]) and absolute (MaxVal - MinVal) variations [37]. The value of 8 was selected empirically as a threshold based on an histogram showing the number of genes as a function of the expression value (MaxVal−MinVal)/(MaxVal/MinVal). Gene Cluster 3.0 allowed us to cluster variable genes using K-Means with the Pearson correlation [37]; they were visualized with Java

| Table 7. Synoptic view of the construction of the transcriptional lacZ fusions strains used in this work. |  |
|-------------------------------------------------------------|
| **Fusion** | **Column 2** | **Column 3** | **Column 4** |
| 2009pRS415 | 3087 | 3089 | 3091 |
| wildtypeZ | 3011 | 3013 | 3015 |
| ndcA::Z | 3079 | 3081 | 3083 |
| nirB::Z | 3065 | 3067 | 3069 |
| Column 2 corresponds to strains carrying plasmids, derived from pRS415 and harboring the different transcriptional lacZ gene fusions used in this work. The strains listed in column 3 and 4 correspond to single copy chromosomal fusion derivatives obtained by phage P1 transduction. The numbers between parentheses refer to the respective recipient and donor strains used for transduction. Four digit numbers refer to strains having JO suffices have been omitted for clarity. |  |  |  |
| HU Regulon |  |  |  |
| Column 2 corresponds to strains carrying plasmids, derived from pRS415 and harboring the different transcriptional lacZ gene fusions used in this work. The strains listed in column 3 and 4 correspond to single copy chromosomal fusion derivatives obtained by phage P1 transduction. The numbers between parentheses refer to the respective recipient and donor strains used for transduction. Four digit numbers refer to strains having JO suffices have been omitted for clarity. |  |  |  |
The determination of the number of clusters was determined by using the iterative criterion of Hartigan:

$$\frac{E_k}{E_{k+1}} - 1 \times (n - K - 1) > 10 \quad [38]$$

Since the statistical distribution of values in the data did not obey the normal law, bootstrap methods provided by Stata Statistical Software R. 9 [91] were used to obtain a more robust non-parametric estimate of the confidence intervals [92]. In order to determine which experimental condition effect (genotype and growth phase) was predominant in each cluster, we performed Kruskall-Wallis non-parametric tests for every condition except hupA vs. hupB. A total of 30 conditions were therefore tested (10 for each phase) to assess the significance of the difference in gene expression between clusters. When the overall test was significant, the genes belonging to the clusters presenting very high mean ranks were considered to be regulated under the given condition. Microarray reproducibility was tested using intra-class coefficients; all Spearman’s rhos were between 0.89 and 0.95 indicating very high data reproducibility. The absolute gene expression values are shown in Supplemental Table 2. For clarity, the individual gene expression levels in Tables 2 to 6 have been normalized by taking, for each growth phase, a value of 1 for the wild-type strain.

Supporting Information

**Figure S1** Comparison of the HU, H-NS, Fis and supercoiling regulons

Found at: doi:10.1371/journal.pone.0004367.s001 (1.55 MB TIF)

**Figure S2** Distribution of the HU regulated operons on the E. coli chromosome.

Found at: doi:10.1371/journal.pone.0004367.s002 (1.64 MB TIF)

**Table S1** Cluster assignment by the Kruskall-Wallis tests.

Found at: doi:10.1371/journal.pone.0004367.s003 (0.05 MB DOC)

**Table S2** Comparison of the RpoS regulon (Saint-Ruf et al, 2004) with the clusters of the HU regulon.

Found at: doi:10.1371/journal.pone.0004367.s004 (0.14 MB DOC)

**Table S3** Genes composing the HU regulon

Found at: doi:10.1371/journal.pone.0004367.s005 (0.92 MB DOC)

**Table S4** Operons composing the HU regulon

Found at: doi:10.1371/journal.pone.0004367.s006 (0.32 MB DOC)

**Table S5** Chaperone and stress functions in the HU regulon.

Found at: doi:10.1371/journal.pone.0004367.s007 (0.27 MB DOC)

**Table S6** Comparison of the genes regulated by DNA supercoiling by Blot et al (2006) (1) and Peter et al (2004) (2).

Found at: doi:10.1371/journal.pone.0004367.s008 (0.08 MB DOC)

**Table S7** Comparison of the genes regulated by HU (1) and by DNA supercoiling by Blot et al (2006) (2).

Found at: doi:10.1371/journal.pone.0004367.s009 (0.03 MB DOC)

**Table S8** Comparison of the genes regulated by HU (1) and by DNA supercoiling by Peter et al (2004) (2).

Found at: doi:10.1371/journal.pone.0004367.s010 (0.04 MB DOC)

**Table S9** Comparison of the genes regulated by HU (1) and by DNA supercoiling by Blot et al (2006) (2) and Peter et al (2004) (3).

Found at: doi:10.1371/journal.pone.0004367.s011 (0.03 MB DOC)

**Table S10** Comparison of the genes regulated by H-NS (1) and by DNA supercoiling by Blot et al (2006) (2).

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**Table S11** Comparison of the genes regulated by H-NS (1) and by DNA supercoiling by Peter et al (2004) (3).

Found at: doi:10.1371/journal.pone.0004367.s013 (0.09 MB DOC)

**Table S12** Comparison of the genes regulated by FIS (1) and by DNA supercoiling by Blot et al (2006) (2).

Found at: doi:10.1371/journal.pone.0004367.s014 (0.07 MB DOC)

**Table S13** Comparison of the genes regulated by FIS (1) and by DNA supercoiling by Peter et al (2004) (3).

Found at: doi:10.1371/journal.pone.0004367.s015 (0.10 MB DOC)

**Table S14** Comparison of the genes regulated by FIS (1) and by DNA supercoiling by Blot et al (2006) (2) and Peter et al (2004) (3).

Found at: doi:10.1371/journal.pone.0004367.s016 (0.03 MB DOC)

**Table S15** Comparison of the genes regulated by HU (1) and by H-NS (Blot et al, 2006 (2)).

Found at: doi:10.1371/journal.pone.0004367.s017 (0.14 MB DOC)

**Table S16** Comparison of the genes regulated by HU (1) and by FIS (Blot et al, 2006 (2)).

Found at: doi:10.1371/journal.pone.0004367.s018 (0.16 MB DOC)

**Table S17** Comparison of the genes regulated by H-NS (1) and FIS (Blot et al, 2006 (2)).

Found at: doi:10.1371/journal.pone.0004367.s019 (0.22 MB DOC)

**Table S18** Comparison of the genes regulated by HU (1), H-NS (2) and FIS (3) (Blot et al, 2006).

Found at: doi:10.1371/journal.pone.0004367.s020 (0.07 MB DOC)

**Table S19** Comparison of the HU regulon with the genes located in the chromosomal areas exhibiting maximum DNA distortion reported by Pedersen et al, (2000).

Found at: doi:10.1371/journal.pone.0004367.s021 (0.06 MB DOC)

**Table S20** Oligonucleotides used for PCR amplification.

Found at: doi:10.1371/journal.pone.0004367.s022 (0.03 MB DOC)

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