

Nucleoid remodeling by an altered HU protein: Reorganization of the transcription program

Sudeshna Kar, Rotem Edgar, and Sankar Adhya*

Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892-4264

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Bacterial nucleoid organization is believed to have minimal influence on the global transcription program. Using an altered bacterial histone-like protein, HU α , we show that reorganization of the nucleoid configuration can dynamically modulate the cellular transcription pattern. The mutant protein transformed the loosely packed nucleoid into a densely condensed structure. The nucleoid compaction, coupled with increased global DNA supercoiling, generated radical changes in the morphology, physiology, and metabolism of wild-type K-12 *Escherichia coli*. Many constitutive housekeeping genes involved in nutrient utilization were repressed, whereas many quiescent genes associated with virulence were activated in the mutant. We propose that, as in eukaryotes, the nucleoid architecture dictates the global transcription profile and, consequently, the behavior pattern in bacteria.

bacterial HU | nucleoid condensation | virulence

The highly organized eukaryotic chromosome requires elaborate remodeling for coordination of transcription processes. The bacterial chromosome, however, constitutes a comparatively open and expanded structure, accessible throughout the cell cycle to DNA-binding proteins, polymerases, and ribosomes (1, 2). The apparent lack of a systemic hierarchy of nucleoid organization in bacteria was attributed to a low stability of histone-like protein–DNA complexes and the dynamic nature of the bacterial chromosome (3). In the absence of any perceptible higher-order chromosome organization imposing a general level of restriction on the accessibility of bacterial promoters, gene expression is believed to be regulated by operon-specific factors, adjacent DNA control elements and local DNA architecture; global nuclear organization is thought to contribute minimally to overall control of cellular processes involving DNA as substrate (4). Considering the exquisite precision with which bacteria can modulate their gene-expression profile to various environmental challenges, the ostensible lack of influence of chromosome organization over global gene regulation is surprising.

Based on its small size, basic nature, cellular abundance, and sequence-independent DNA-binding capacity, the nucleoid-associated protein HU has long been characterized as the bacterial counterpart of eukaryotic histones (5). HU was initially attributed with the capability to form nucleosome-like structures in bacterial chromosomes (6), but subsequent studies have resulted in conflicting reports about the exact role of HU in chromosome compaction (7, 8). In almost all bacteria except enterobacteriaceae, including *Escherichia coli*, HU exists as an 18-kDa homodimer. In *E. coli*, HU is a heterodimer of two subunits, HU α and HU β .

Using a gain-of-function HU α mutant, we demonstrate that nucleoid structural reorganization in bacteria can directly induce a radical change in the gene-expression profile, resulting in dramatic changes in cellular morphology and physiology.

Materials and Methods

Bacterial Strains, Media, and Growth Conditions. Mutagenesis of HU α and integration of the mutant *hupA* gene into the chromosome have been described in ref. 9. A spectinomycin-resistance cassette was used as a marker in both the plasmid and

chromosomal constructs of the *hupA* mutant. Although the spectinomycin-resistance marker, on its own, did not show any of the phenotypes displayed by HU α ^{E38K.V42L}, there was a sharp reduction in the mutant HU α expression when some other selective markers were used as substitutes, the basis of which is not known. For creating pHU-GFP6, *hupA* was amplified with the primer set CGAAGCTTATGAACAAGACTAACT-GATTG (HindIII-HU forward) and CCACCGGTTTAACT-GCGTCTTTCAGTGC (AgeI-HU reverse). The amplified DNA product, which lacked the last four nucleotides of the *hupA* gene, was cleaved and cloned between the HindIII and AgeI sites of plasmid pGFPuv (Clontech), creating a HU α –GFPuv translational fusion. For plasmid pPROU37, the *proU* promoter was amplified as a 612-bp fragment (–240 to +372) by using PCR primers with 5' EcoRI and 3' PstI restriction sites and cloned between the EcoRI and PstI sites of plasmid pSA850, which contains the ρ -independent transcription terminator immediately downstream of the PstI site. LB containing 0.4% glucose was used for growth experiments. The metabolic profile of the wild-type and mutant strains was assayed by using GN2 plates (BioLog Chemical, San Diego).

Nucleoid Isolation and Reassembly with HU. Intact nucleoids were extracted from strain DM0100 (Δ *hupAhupB*), following the procedure of Zimmerman and Murphy (10). Isolated nucleoids were incubated with wild-type HU α and HU α mutant at a (HU α)₂:nucleoid DNA molar ratio of 4 nM:0.3 fM in a buffer containing 10 mM Tris (pH 8.0) and 50 mM KCl for 10 min on ice. DAPI was added to a final concentration of 5 μ g/ml, and the resultant nucleoids were visualized by fluorescence microscopy.

Fluorescence Microscopy. Mutant *E. coli* cells with pHU–GFPuv were grown at 37°C until OD_{600 nm} 0.5 and induced with 1 mM isopropyl β -D-thiogalactoside (IPTG). Samples were taken out at various times and stained with *N*-(3-triethylammoniumpropyl)-4(6-(4(diethylamino)phenyl)hexatrienyl)pyridinium dibromide (FM 4–64) for the visualization of the lipid membrane. Microscopy was performed on an Eclipse E1000 microscope (Nikon) with a Sencam QE charge-coupled-device camera (Cooke, Romulus, MI) controlled by IP Labs software (Scanalytics, Rockville, MD) with filter sets for DAPI or differential interference contrast.

Other experimental methods are described in *Supporting Text*, which is published as supporting information on the PNAS web site.

Results

Changes in Colony Architecture and Cell Morphology by an HU α Mutant. While performing mutagenesis of the *hupA* gene in *E. coli* K-12 strain MG1655 to identify HU α mutants specifically defective in DNA looping involved in the transcription repres-

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Abbreviation: IPTG, isopropyl β -D-thiogalactoside.

*To whom correspondence should be addressed at: Laboratory of Molecular Biology, National Cancer Institute, 37 Convent Drive, Room 5106, Bethesda, MD 20892-4264. E-mail: sadhya@helix.nih.gov.

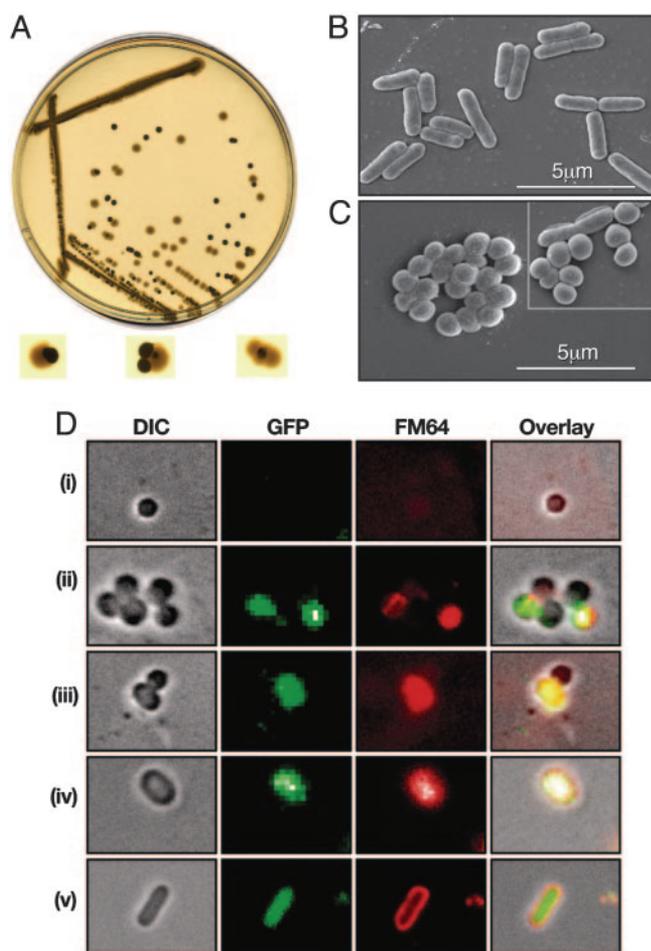


Fig. 1. Effect of $HU\alpha^{E38K,V42L}$ substitution on colony morphology and cell architecture. (A) Conversion of colony morphology of wild-type *E. coli* cells carrying the mutant *hupA* plasmid, upon losing the mutant clone, on nonselective plates. (B and C) Scanning electron micrographs of wild-type *E. coli* (B) and *hupA* mutant (C) cells. (Inset) Revertant cells from regions of papillae around the *hupA* mutant colonies. (D) Conversion of cellular morphology of *hupA* mutant upon induction of plasmid-borne wild-type $HU\alpha$ -GFP. Cell shape, GFP fluorescence, membrane stain, and overlay of all three fields at 0 (i), 45 (ii), 90 (iii), 135 (iv), and 180 min (v) after the addition of IPTG. DIC, differential interference contrast microscopy.

sion of the gal operon (9), we encountered a chromosomal mutant which superrepressed the gal transcription *in vivo*. The mutant $HU\alpha$ had two amino acid substitutions, E38K and V42L. The $HU\alpha^{E38K,V42L}$ mutant displayed changes in its phenotype, as described below. We cloned the *hupA* gene encoding $HU\alpha^{E38K,V42L}$ with its own promoter, along with a spectinomycin-resistance cassette engineered as an adjacent selective marker into a plasmid and introduced it into a $\Delta recA$ *E. coli* strain. Based on colony morphology, two kinds of transformants were obtained. Some were flat and translucent with irregular edges, resembling the parental wild-type characteristic. Others appeared to be opaque, glossy, and round, with smooth edges. When the transformants belonging to the second class were grown on nonselective LB plates, the smooth, dense colonies segregated into the rough, translucent form (Fig. 1A). Restreaking on selective and nonselective plates and plasmid extraction from the two kinds of colony forms showed that the round, smooth colonies emerged as a result of the plasmid-borne mutant *hupA* gene and, upon the loss of the plasmid, the cells reverted back to their normal colony morphology.

The mutant *hupA* gene was transferred to the chromosome of the wild-type *E. coli* strain MG1655 at the *hupA* locus (strain SK3842). The chromosomal *hupA* mutant also had dense, smooth, round colonies. The mutant *hupA* phenotype was epistatic to the wild-type *hupB* allele. Transmission electron microscopy of SK3842 showed that the mutant cells had lost the elongated, rod-shaped appearance that is characteristic of wild-type *E. coli* (average length $2.45 \pm 0.53 \mu\text{m}$) and assumed a diminutive coccoid morphology (average length $0.78 \pm 0.37 \mu\text{m}$) (Fig. 1B and C). Cells from papillae generated around the mutant colonies on plates frequently included phenotypic revertants that resembled the wild-type cell (Fig. 1C Inset). The E38K, V42L change appeared to be gain-of-function mutations; deletion of the mutant *hupA* allele or replacement of it with the wild-type allele restored normal morphology (data not shown).

To show that the morphological changes exhibited by SK3842 were a direct consequence of $HU\alpha^{E38K,V42L}$ expression, and the effects could be reversed by complementation with a large excess of wild-type $HU\alpha$, we introduced a multicopy plasmid containing an inducible wild-type $HU\alpha$ -GFP fusion into the mutant strain. In the absence of $HU\alpha$ -GFP expression, the mutant cells remained coccoid and were poorly stained by the membrane stain FM-64 (Fig. 1Di). Upon induction with IPTG, some of the coccoid cells began to express the $HU\alpha$ -GFP fluorescence and, concomitantly, incorporate the membrane stain (Fig. 1Dii), indicating a shift toward normalcy in the membrane architecture. With continued induction of the wild-type $HU\alpha$ fusion protein, some of the spherical mutant cells started to adopt a slightly elongated ovoid shape, both in dividing (Fig. 1Diii) and resting (Fig. 1Div) cells. The GFP fluorescence in these transitional cells confirmed that the morphological transformations were not random occurrences but effected by the overexpression of the wild-type $HU\alpha$ fusion. Finally, these ovoid cells changed into regular elongated rods, characteristic of wild-type *E. coli* (Fig. 1Dv).

Improved Growth Characteristics and a Wider Temperature-Adaptability Profile of the *hupA* Mutant Cells. In rich media, the final cell yield of SK3842 at 37°C was significantly higher ($\approx 30\%$) than the wild type (Fig. 2A). This difference in the final cell density was even more striking at nonoptimal temperatures. At both 15°C and 47°C, the mutant reached high cell concentrations, whereas the wild type was severely crippled in its ability to achieve healthy cell growth. The growth rate of the mutant (2.6 generations per h) was also notably higher than that of the wild type (2.43 generations per h) at 37°C (Fig. 2B). The difference in the growth rate was also more pronounced at lower temperatures. But, at temperatures above 42°C, the growth rate of the mutant was actually lower than that of the wild type, in spite of the mutant's higher final cell density.

The Mutant Strain Exhibited Altered Anabolic and Catabolic Potential. Investigation of the nutritional requirements showed that the mutant was prototrophic for only the amino acids aspartate, glutamate, asparagine, glutamine, cysteine, lysine, alanine, and tryptophan. The mutant also had a requirement for nicotinic acid for growth in minimal media. The mutant *hupA* allele also had pronounced pleiotropic effects on carbon utilization, as determined by its ability to grow on different substrates in BioLog plates (Fig. 2C and D). SK3842 showed little or no growth on many sugars (galactose, lactose, arabinose, melibiose, fucose, and rhamnose), TCA cycle intermediates (succinate, *cis*-aconate and α -ketoglutarate), amino acids (D- and L-alanine, asparagines, and aspartate), fermentation acids (formate and acetate), and short-chain fatty acids (α -hydroxybutyric acid). However, the mutant showed enhanced growth on glucose, fructose, and mannose; polyols (glycerol and mannitol); hexonates and hexuronates (gluconate, glucuronate, and galactur-

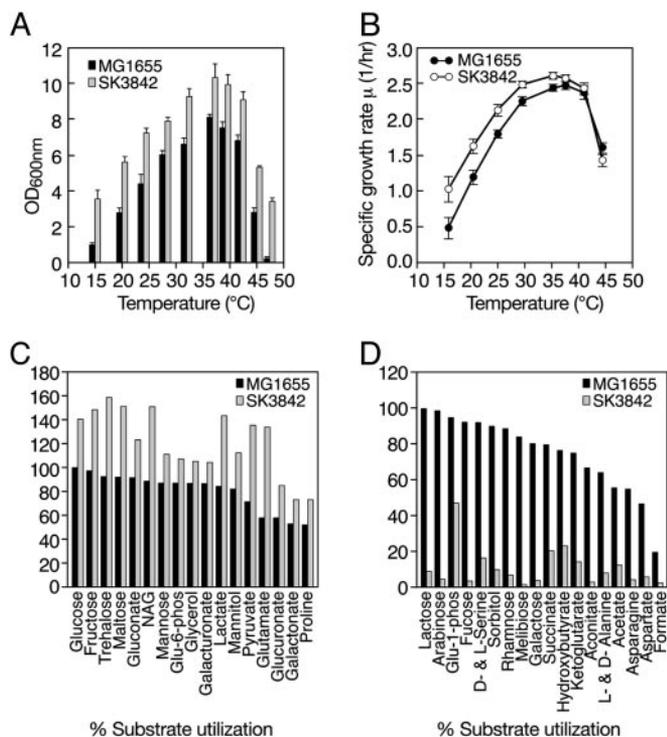


Fig. 2. Effect of HU $\alpha^{E38KV42L}$ substitution on growth and metabolism. (A) Saturation cell densities of the wild type and *hupA* mutant at different temperatures. (B) Specific growth rates of the wild type and *hupA* mutant at different temperatures. (C and D) Differences between the wild type and *hupA* mutant in utilization of individual substrates as sole carbon source. (C) Substrates that were used better by the *hupA* mutant. Growth ($A_{600\text{nm}}$) of the wild type on glucose was arbitrarily set at 100%, and all other values for growth on different substrates were expressed as percentages relative to growth of the wild type on glucose. (D) Substrates that were used poorly by the *hupA* mutant. Growth ($A_{600\text{nm}}$) of the wild type on lactose was arbitrarily set at 100%, and all other values were expressed as relative percentages.

onate); fermentation acids (pyruvate and lactate); and proline, glutamate, and trehalose.

Quiescent Virulence Genes Were Activated in the Mutant. A number of genes that are quiescent under normal laboratory growth conditions were expressed in the mutant. These include genes coding for dormant virulence factors like hemolysin and curli fibers. The hemolysin encoded by the *hlyE* gene of *E. coli* K-12 is a cytolytic protein that causes lysis of mammalian cells by pore formation (11). Curli fibers are thin, aggregative surface structures expressed during mammalian infection and promote formation of biofilms (12). Unlike the wild type, SK3842, when cultured on blood agar plates, showed a clear zone of hemolysis, indicating the expression of the hemolysin by the mutant strain (Fig. 3A). The zone of hemolysis became wider and more prominent when the blood agar plates were supplemented with 5 $\mu\text{g/ml}$ nicotinamide. Quantitative liquid hemolytic assays confirmed that the hemolysin activity in SK3842 was induced to a significant level, which was further enhanced by nicotinamide supplementation (Fig. 3C). SK3842 also grew as deep red colonies on Congo red plates at 37°C, indicating that the mutant strain was expressing curli fibers whereas the wild-type cells were not (Fig. 3B). Because expression of curli fibers is also a strong indicator of biofilm formation, we assessed the potential for biofilm formation by the mutant. Fig. 3D shows that the biofilm formation was much more extensive in the mutant than in the wild type.

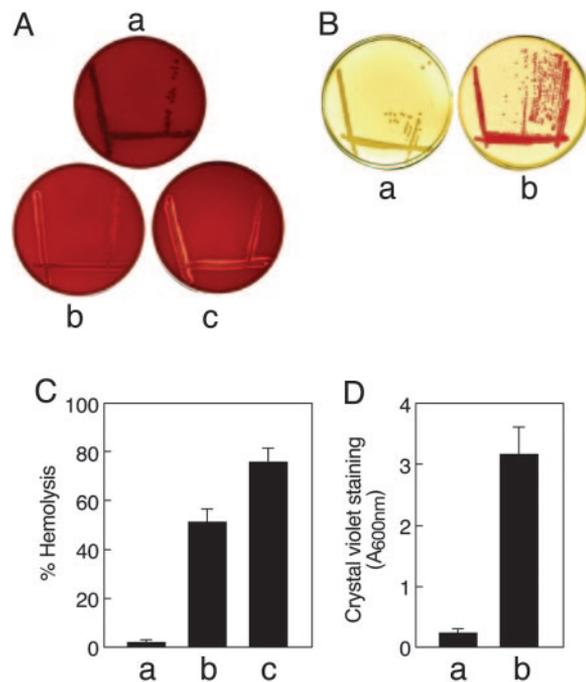


Fig. 3. Expression of quiescent virulence genes in the *hupA* mutant. (A) Expression of hemolysin by wild type (a), *hupA* mutant (b) and *hupA* mutant supplemented with nicotinamide (c) on blood agar plates. (B) Curli-fiber production by wild type (a) and *hupA* mutant (b) on Congo red plates. (C) Quantitative hemolytic activity of wild type (a), *hupA* mutant (b), and *hupA* mutant supplemented with nicotinamide (c), as quantitated by lysis of sheep erythrocytes by culture supernatants. (D) Biofilm production in the wild type and *hupA* mutant, as measured by crystal violet staining of adhering cells on polystyrene microtitre plates.

Condensation of the Bacterial Nucleoid by HU $\alpha^{E38K,V42L}$. *E. coli* mutants with coccoid morphology (e.g., *rodA*, *pbpA*, and *mreBCD*) are normally defective in growth, cell division, and nucleoid segregation. However, phase-contrast microscopy showed that the mutant cells were remarkably uniform in size and contour, without the appearance of any anucleate, filamentous, or abnormally shaped cells that are usually associated with aberrant cell division and chromosome segregation (Fig. 4B). Most of the cells were engaged in active cell division, reflecting the robust growth rate of the mutant. Consistent with the markedly reduced cell volume of the mutant, the nucleoids appeared to be much more condensed, in comparison with that of the wild type (Fig. 4A).

Because HU is a major architectural component of the bacterial nucleoid (13), we compared the nucleoid configuration in the wild type and mutant by transmission electron microscopy on thin sections of actively growing cells. The wild-type *E. coli* nucleoid, as expected, was spread out as a dispersed, lobular structure interspersed with the ribosome-filled, granular cytoplasm (Fig. 4C Upper). In contrast, the nucleoid in SK3842 was organized into a densely condensed unit of tightly coiled DNA packed into a limited space (Fig. 4C Lower). There was a highly condensed, electron-dense central core with coils and loops of exposed DNA strands arranged in a rosette-shaped structure. Nucleoid condensation was visible during all stages of the cell cycle, from a newly divided cell to a resting cell.

The changes encountered in the mutant could be a result of higher levels of the mutant HU α . Western blot analysis of cellular lysates from log-phase cultures of wild type, $\Delta hupB$, and mutant *hupA*($\Delta hupB$) showed that the amount of HU α in all strains was almost identical (Fig. 4D). To investigate whether the nucleoid condensation observed in the mutant was a direct effect

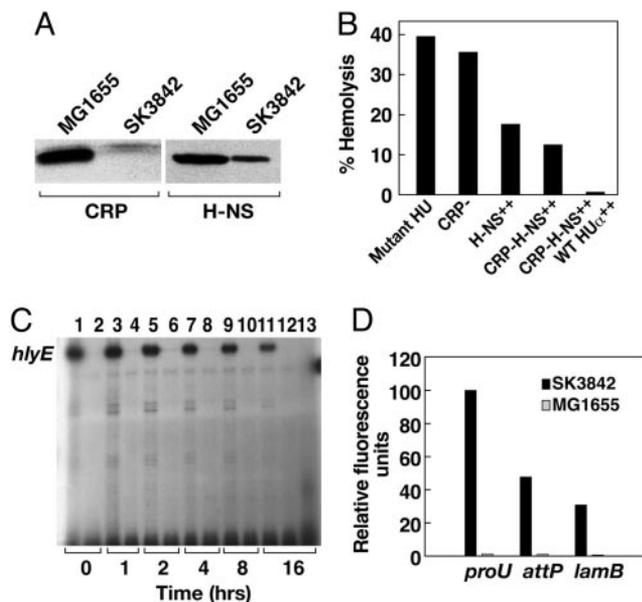


Fig. 6. Direct influence of $HU\alpha^{E38K,V42L}$ -mediated nucleoid reorganization on specific changes in transcription profile. (A) Western blot analysis of CRP and H-NS concentration in midlog-phase cultures of wild-type and *hupA* mutant cells. (B) Hemolytic activity of *hupA* mutant strains with altered levels of transcription regulators for *hlyA*. From left to right, the strains used were SK3842, SK3842 Δcrp , SK3842 (pHNS42), SK3842 Δcrp (pHNS42), and SK3842 Δcrp (pHNS42 and pHU-GFPuv). Assays were done 16 h after the addition of IPTG. (C) Primer extension analysis of *hlyE* mRNA expression in SK3842 Δcrp pHNS42 (lanes 1, 3, 5, 7, 9, and 11) and MG1655 Δcrp pHNS42 (lanes 2, 4, 6, 8, 10, and 12) at different time points after induction of H-NS. Lane 13 shows the *hlyE* mRNA expression in SK3842 Δcrp pHNS42, pHU-GFPuv at 16 h after IPTG addition. (D) GFP fluorescence intensities from P_{proU} -GFP translation fusions at the *proU*, *attP*, and *lamB* chromosomal loci in the wild-type and *hupA* mutant strains.

slowly on the gel. Plasmid from the mutant strain appeared to have significantly higher negative supercoiling in comparison with that from the wild type (Fig. 5D).

Nucleoid Reorganization Had a Direct Impact on Transcription Pattern.

The effect of $HU\alpha^{E38K,V42L}$ on the changes in the transcription profile could be mediated (i) directly, by remodeling the three-dimensional architecture of the nucleoid, thereby changing the susceptibility of individual promoters to the transcription machinery; (ii) indirectly, by modulating the expression of a subset of global regulatory factors that leads to the transcription reorganization; or (iii) through a combination of these two effects. The transcription of *hlyE* in wild-type *E. coli* is strictly contingent upon two regulators, the presence of the activator CRP and the absence of the repressor H-NS (16). Western blot analysis showed that, compared with the wild type, the level of CRP in the mutant was drastically diminished (Fig. 6A). The amount of H-NS was also reduced, but not totally eliminated, in the mutant. To corroborate the influence of nucleoid reorganization on the altered transcription pattern, we measured the expression of the *hlyE* gene in the context of no transcription-activator protein, excess transcription-repressor protein, and a combination of both (Fig. 6B). In the absence of cAMP (Δcrp), hemolysin expression in the mutant was similar to that in the wild type, confirming that, contrary to the wild-type situation, cAMP-CRP does not play a major role in the constitutive expression of *hlyE* in the mutant. In SK3842 bearing a multicopy plasmid expressing H-NS, even overnight induction of H-NS could reduce the level of hemolysin to only $\approx 50\%$ of that of SK3842, showing that H-NS, which causes total silencing of *hlyE* under

normal circumstances, failed to turn off transcription, even at excess concentrations. In the *hupA* mutant with both *crpA* deletion and over-expressing H-NS, the level of hemolysin was reduced slightly more but was not turned off totally. However, when provided with excess wild-type $HU\alpha$ from a multicopy plasmid, the *hlyE* gene was completely repressed. We performed primer-extension studies on RNA extracted from the *hupA* (Δcrp) mutant carrying the plasmid pHNS42 to study the expression of *hlyE* (Fig. 6C). Consistent with the liquid-hemolysin assay result, the expression of *hlyE* decreased gradually with time and reached a plateau at $\approx 50\%$ of its original level. Expression of the wild-type $HU\alpha$, however, completely turned off *hlyE* transcription. Although the stimulatory effect of $HU\alpha^{E38K,V42L}$ on *hlyE* was rather independent of transcription regulators, we envision that the overall effect of $HU\alpha^{E38K,V42L}$ on most promoters would be more complex and ramified. Because global transcription regulators are a part of the cellular transcriptome, their expression levels would also be affected significantly in the mutant and contribute to the overall changes in transcription pattern.

If the nucleoid architecture in the mutant determined the fate of individual promoters, at least partially, then the context of chromosomal location should also play a role in determining the sensitivity of individual promoters. We inserted a P_{proU} -*gfp* translational fusion at different chromosomal locations in the wild type and mutant, replacing the existing native promoters. In the mutant, P_{proU} activity was highest at its original chromosomal locus, decreased by $\approx 50\%$ at the *attP* locus, and decreased even further at the *lamB* locus (Fig. 6D). P_{proU} was repressed at all three loci in the wild-type strain. The differential activity of a single promoter at different chromosomal locations further confirmed that the global topological state of the chromosome determined the expression of individual genes.

Discussion

Nucleoid Condensation and Global Changes in Gene Expression by a Mutant $HU\alpha$.

We describe a mutant bacterial histone-like protein, $HU\alpha^{E38K,V42L}$, which profoundly affected the morphology, growth, and physiology in wild-type *E. coli*. $HU\alpha^{E38K,V42L}$ and made the bacterial nucleoid undergo a radical transformation from a loosely organized structure into a densely condensed globular configuration, without disruption of growth, chromosome segregation, and cell division. Overexpression of some histone-like proteins is known to induce strong chromosomal condensation in *E. coli*. Cyt1Aa of *Bacillus thuringiensis* (17), chlamydial histone H1-like protein, Hc1 (18), or *E. coli* nucleoid protein H-NS (19), when overexpressed, causes a global downshift in transcription and even cell death. The nucleoid condensation by the Dps protein during stationary phase (20) or the absence of the SeqB protein in wild-type *E. coli* (21) causes growth and metabolic downshifts. Condensed nucleoids are not always symbolic of functional inefficiency; the naturally occurring, highly compacted nucleoids of *Deinococcus* (22) or the metabolically active, condensed nucleoids induced by certain mitochondrial and chloroplast proteins (23, 24) are prime examples. The *hupA* mutant also exhibited an increase in DNA supercoiling *in vivo*. DNA supercoiling is known to be modified during many environmental challenges, including host infection, thereby coordinating the outputs of the gene-regulatory networks in response to environmental cues (25). Hence, the change in degree of global supercoiling in the *hupA* mutant was not unexpected, given the widespread changes in its transcription program. It is not clear whether the increased superhelicity was generated because of changes in cellular topoisomerase activities or an altered mode of supercoil constraint by the mutant $HU\alpha$. It is, however, clear that the combination of $HU\alpha^{E38K,V42L}$ -mediated chromosome compaction and altered superhelical

tension resulted in unique functional remodeling of the nucleoid structure, causing a global shift in the transcription pattern.

HU α ^{E38K,V42L}-Mediated Cellular Changes Define a Shift in *E. coli* Behavior. There was a functional directionality in the global changes of the transcription profile of SK3842; many of the genes, which are redundant or unfavorable to *E. coli* K-12 for its laboratory existence, were activated, whereas other genes that are routinely expressed were turned off. The morphogenic differentiation of *E. coli* into coccoid form serves a vital role in its response to the host environment in certain cases. *E. coli* BJ4, during growth in the rat intestine, differentiates from rods to cocci, and the coccoid form is selected for *in vivo* (26). During the multistage differentiation and maturation program *in vivo*, uropathogenic *E. coli* converts from rods to cocci as a natural process of infection (27). The *hupA* mutant was more efficient in using substrates involved in glycolytic and Entner–Doudoroff pathways and the phosphotransferase-uptake systems. Substrates from the tricarboxylic acid cycle, glyoxalate shunt, and gluconeogenesis pathways were used very poorly. *E. coli* utilizes gluconate as the main carbon source and exploits only a small number of other carbohydrates to grow and colonize in the small intestine (28, 29). It appears significant that SK3842 could efficiently metabolize only certain carbon sources, most of which corresponds to the substrates preferred by *E. coli in vivo*. It has also been shown that most of the amino acid biosynthetic genes in *E. coli* are repressed *in vivo* (29). Finally, the extensive biofilm formation in the *hupA* mutant and the expression of hemolysin and curli fibers all point to coordinated and directional changes in the transcription profile of the *hupA* mutant, more suitable for survival inside a host environment.

Normal Architecture and Transcriptional Program in Bacteria. HU α ^{E38K,V42L} brought about a qualitatively different nucleoid configuration that engendered a functional shift in the transcription pattern. The altered nucleoid architecture and the resulting changes in the gene-expression profile could be reversed back to their incipient states by the introduction of a large excess of wild-type HU α , revealing that mutant HU α acted as a master switch to coordinate the entire spectrum of physical and physiological changes. Bacteria can switch their gene-expression program rapidly and precisely in response to environmental changes. We propose that nucleoid structural reorganization could serve as an efficient mechanism to synchronize the genetic response to external conditions. Certain effector molecules present in pathogenicity-inducing environments probably elicit similar changes in nucleoid condensation, leading to swift and concerted changes in the basal transcription program.

The Nature and Possible Evolutionary Significance of HU α ^{E38K,V42L} Mutant. HU α ^{E38K,V42L} had two amino acid substitutions, E38K and V42L, both of which were essential for the phenotypic changes in the *hupA* mutant. Wild-type HU α has a lysine at position 37. So, the presence of lysine at position 38 created a lysine–lysine motif in this highly basic protein. It is noteworthy that K38 is one of the critical amino acids responsible for the thermostability of BstHU (30). We have also observed that chromosomal integration of BstHU into the Δ *hupA E. coli* strain manifested many of the characteristics of SK3842 (S.K. and S.A., unpublished data).

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