Coordination of genomic structure and transcription by the main bacterial nucleoid-associated protein HU

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The histone-like protein HU is a highly abundant DNA architectural protein that is involved in compacting the DNA of the bacterial nucleoid and in regulating the main DNA transactions, including gene transcription. However, the coordination of the genomic structure and function by HU is poorly understood. Here, we address this question by comparing transcript patterns and spatial distributions of RNA polymerase in *Escherichia coli* wild-type and *hupA/B* mutant cells. We demonstrate that, in mutant cells, upregulated genes are preferentially clustered in a large chromosomal domain comprising the ribosomal RNA operons organized on both sides of *OriC*. Furthermore, we show that, in parallel to this transcription asymmetry, mutant cells are also impaired in forming the transcription foci—spatially confined aggregations of RNA polymerase molecules transcribing strong ribosomal RNA operons. Our data thus implicate HU in coordinating the global genomic structure and function by regulating the spatial distribution of RNA polymerase in the nucleoid.

Keywords: RNA polymerase; DNA supercoiling; stable RNA operons; transcription foci; replication origin

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INTRODUCTION

In the classical model organism *Escherichia coli*, the transcriptional regulatory network comprises hundreds of dedicated transcription factors expressed at low levels and binding to a few specific sites located in gene promoter regions. However, a different regulatory effect is exerted by abundant nucleoid-associated proteins (NAPs), the bacterial counterparts of histones, which stabilize long-range structures by cooperative binding of multiple genomic sites (Azam & Ishihama, 1999; Dame, 2005). The unique feature of the regulatory effect of NAPs is in modulating the accessibility of free DNA supercoils to transcription

machinery by modulating the state of DNA compaction (Travers & Muskhelishvili, 2005a). The nucleoprotein structures stabilized by NAPs thus engage neighbouring loci in continuous or ‘analogue’ transcriptional control. This regulation converts the fluctuations in DNA supercoil energy into transcript patterns, as opposed to the ‘digital’ type of control exerted by dedicated regulators targeting a few specific sites (Blot *et al*, 2006; Marr *et al*, 2008). Importantly, analogue control involves cross-talk between NAPs and DNA topoisomerases, adjusting the DNA supercoiling and instant cellular metabolism, and thus optimizing bacterial adaptation (Travers & Muskhelishvili, 2005b).

NAPs have been shown to sustain supercoiling homeostasis, and to organize dynamic topological domains and spatial transcript patterns in the genome (Hardy & Cozzarelli, 2005; Blot *et al*, 2006; Marr *et al*, 2008). The DNA binding preferences and structures stabilized by the main NAPs differ (Azam & Ishihama, 1999; Pinson *et al*, 1999; Dame *et al*, 2001). The global repressor H-NS (histone-like nucleoid structuring protein) stabilizes tight plectonemic DNA interwindings consistent with silencing (Dorman, 2004; Maurer *et al*, 2009), whereas the main NAP HU (histone-like protein)—which is a heterodimer of HU α and HU β proteins in *E. coli*—constrains dynamic toroidal supercoils supporting transcription (Rouviere-Yaniv *et al*, 1979; Broyles & Pettijohn, 1986).

A previous study implicated HU in replication initiation and chromosomal partitioning (Jaffe *et al*, 1997). Initiation of *OriC* replication was shown to require both HU and high negative superhelicity (Dixon & Kornberg, 1984; Crooke *et al*, 1991). Recent studies have revealed alterations of the nucleoid condensation and transcription in a gain-of-function HU α mutant (Kar *et al*, 2005), and also a role for the supercoil-constraining capacity of HU in gene regulation (Oberto *et al*, 2009). However, a clear understanding of the impact of HU in coordinating genomic structure and function is still lacking. In this study, we address this question by investigating the transcript patterns and distributions of RNA polymerase (RNAP) molecules in nucleoids of wild-type and *hupA/B* mutant *E. coli* cells.

RESULTS

Using DNA microarrays, we compared the genomic expression patterns of *E. coli* CSH50 wild-type and *hupA/B* mutant strains.

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Fig 1 | Spatial reorganization of transcription in cells lacking the histone-like protein HU. (A) Frequency distribution of gene transcripts upregulated in wild-type (grey) and *hupA/B* (black) backgrounds. (B) Frequency distributions of upregulated transcripts associated with DNA relaxation (grey) and high negative supercoiling (blue) in wild-type and *hupA/B* mutant cells (black and red, respectively). (C) Genomic distribution of gyrase-binding sites (Jeong *et al*, 2004). All frequency distributions were obtained by averaging, using a sliding window of 200 ORFs. Chromosomal positions of ribosomal RNA operons, *OriC* and *Ter* are indicated. (D) Dependence of cruciform formation (arrows) in (AT)₃₄ reporter plasmids on the superhelical density (σ) *in vitro* (left panel, lanes 1–8). The indicated σ values are within 15% of precision. Detection of cruciforms in exponentially growing wild-type and *hupA/B* mutant cells (right panel). 0.0, fully relaxed DNA template; oc, open circular form; ORFs, open reading frames; *OriC*, replication origin; *rrn*, ribosomal RNA operons; *Ter*, replication terminus; wt, wild type.

Notably, while the growth defect observed in the *hupA/B* mutant depends on the growth medium and oxygen availability (Oberto *et al*, 2009), under our conditions cells grew at similar rates, but the mutant demonstrated a prolonged lag phase (data not shown). Clustering of the differentially expressed genes in gene ontology (GO) groups revealed that, despite differences in experimental conditions and applied analyses, affected functions are largely consistent with a recent report (Oberto *et al*, 2009), that indicated a global reorganization of transcription (supplementary Tables S1 and S2 online). However, the genomic distribution of differentially expressed genes also revealed a striking spatial asymmetry. In a wild-type background, upregulated genes—that is, genes with positive log ratios—were predominantly clustered in a large chromosomal region comprising the replication terminus (*Ter*). However, upregulated genes in the mutant were clustered in a comparable region comprising *OriC* with ribosomal RNA operons (*rrn*), and were apparently delimited by distal operons *rrnG* and *rrnH* on both replichores (Fig 1A). We ruled out the effects of gene dosage on the observed pattern because, consistent with previous findings (Jaffe *et al*, 1997), the *OriC/Ter* DNA ratios in wild-type and *hupA/B* mutant cells were similar (supplementary Fig S1 online).

Analyses of the supercoiling dependence of the transcripts using a published data set (Blot *et al*, 2006) showed that *hupA/B* mutant upregulated genes preferentially use high negative superhelicity (Fig 1B; it should be noted that for technical reasons *rrn*

genes are not included in our transcript patterns). Importantly, this pattern correlated with genomic distribution of DNA-binding sites for gyrase, the main enzyme introducing negative supercoils into the DNA (Fig 1C; Jeong *et al*, 2004).

As on prolonged cultivation, the cells lacking HU can acquire suppressor mutations in gyrase genes (Malik *et al*, 1996), we sequenced *gyrA/B* genes in *hupA/B* mutant and isogenic CSH50 wild-type parent, and also in the wild-type *E. coli* W3110 strain. In all three backgrounds, we found a C-to-G transversion at position 1,153 of the *gyrB* ATPase domain, substituting Pro 385 by alanine (data not shown). We believe this substitution represents a natural variation and consider it irrelevant to our observations. The cellular levels of GyrA and GyrB proteins in wild-type and mutant cells were also similar (supplementary Fig S2 online).

Our observation that upregulated genes in the *hupA/B* mutant preferentially use high negative superhelicity was intriguing because in this mutant the DNA-relaxing activity was seen to have increased in parallel with reduced overall negative superhelicity (Bensaid *et al*, 1996; Malik *et al*, 1996). However, whether the unconstrained supercoiling is also altered in the *hupA/B* mutant remains unknown. To clarify this point, we used plasmid constructs containing 34 bp AT tracts, adopting cruciform geometries under conditions of high negative superhelicity, as reporters to measure unconstrained negative supercoiling *in vivo* (Bowater *et al*, 1994). Probing of (AT)₃₄ cruciforms *in vitro* over a range of superhelical densities (σ) by potassium permanganate

Fig 2 | Exponentially growing *hupA/B* mutant cells are impaired in foci formation. (A) Representative fluorescence microscopy images of wild-type cells showing RNAP-dense areas (foci) at the poles of the nucleoid (white arrowheads; only two positions are indicated). (B) Representative image of a *hupA/B* mutant cell. It should be noted that RNAP is more evenly distributed in the nucleoid. Fluorescence images of RpoC-YFP (RNAP), DRAQ5 (nucleoid) and phase-contrast images are shown. In the merged images, green is the RpoC-YFP fluorescence signal, and red is the DRAQ5 fluorescence signal. Scale bars, 2 μ m. RNAP, RNA polymerase; YFP, yellow fluorescent protein.

reactivity assay showed a sharp transition in local DNA geometry between $\sigma = -0.034$ and $\sigma = -0.047$, and cruciform stabilization at $\sigma > -0.047$ (Fig 1D). *In vivo*, the (AT)₃₄ cruciform formation could be detected in both *hupA/B* mutant and wild-type cells, indicating high levels of free superhelicity in both cases (σ at least > -0.034 , as approximated from our *in vitro* data; Fig 1D). Thus, although global negative superhelicity is reduced in the *hupA/B* mutant (Malik *et al*, 1996), a high level of free superhelicity is sustained that is consistent with homeostatic regulation (Blot *et al*, 2006). These findings suggest a global readjustment of nucleoid structure and transcription in the *hupA/B* mutant.

The most prominent structures associated with bacterial transcription are the transcription foci, representing local accumulations of RNAP transcribing the exceptionally strong ribosomal RNA (rRNA) operons (Cabrera & Jin, 2003, 2006). To test whether the spatial reorganization of transcription in the *hupA/B* mutant had any effect on the formation of transcription foci, we fused the *rpoC* gene encoding the β -subunit of RNAP with a *yfp* (yellow fluorescent protein) gene to produce a fluorescent protein and substituted the *rpoC* allele in both the wild-type and *hupA/B* cells. Laser scanning microscopy investigations of RpoC-YFP signal distribution in nucleoids revealed a regular pattern of transcription foci located close to nucleoid poles in exponentially growing wild-type cells (Fig 2A; supplementary Fig S3A online). By contrast, in *hupA/B* cells the RpoC-YFP signal was distributed more evenly throughout the nucleoid (Fig 2B; supplementary Fig S3B,C online). Unlike the HU mutant, cells lacking either H-NS, DNA protection during starvation or integration host factor were proficient in transcription foci formation (supplementary Fig S3D–F online). Unfortunately, we could neither delete the *fis* gene in the *rpoC-yfp* background, nor introduce the *rpoC-yfp* allele in a *fis* mutant. However, mutations of the main NAPs, including factor for inversion stimulation, did not induce transcription asymmetry similar to *hupA/B* (Blot *et al*, 2006; G.M., unpublished data).

As the transcription asymmetry features a large region comprising OriC and rRNA operons, whereas the transcription

foci confine these operons, we asked whether the transcription foci are associated with OriC. We used *lac* operator arrays inserted in the vicinity of either OriC or Ter as markers, and visualized them by expressing a fluorescent Lac repressor-CFP (cyan fluorescent protein) fusion from the pLAU53ÄtetR plasmid construct (Lau *et al*, 2003). These cells also carried a chromosomal *rpoC-yfp* allele, which enabled us to co-localize the LacR-CFP and RpoC-YFP signals in the nucleoid. We found that in exponentially growing wild-type cells the pattern of RpoC-YFP signals was similar to that of OriC, but not of Ter (compare Fig 3A and Fig 3B). From these data, we infer that the OriC and transcription foci are located in close vicinity in wild-type cells, whereas in the *hupA/B* mutant, which exhibits a more even distribution of RNAP in the nucleoid, this organizational capacity is lost (Fig 2B; supplementary Fig S4 online).

DISCUSSION

Here, we show that the formation of transcription foci as confined structures of the nucleoid is dependent on HU, and the absence of foci in the *hupA/B* mutant correlates with a spatially rearranged transcript pattern. The boundaries of this pattern closely coincide with distal *rrn* operons on both replichores, delimiting a chromosomal domain of >2 Mb in extent, which we denote as the *rrn* macrodomain for convenience. The spatial organization of the *rrn* macrodomain is in keeping with chromosomal macrodomains identified previously by an approach based on measurements of site-specific recombination events in the chromosome (Valens *et al*, 2004). An initial estimate is that the extent of the *rrn* macrodomain corresponds to the previously defined OriC macrodomain, including either large parts or most of the two flanking less-structured domains (Fig 4A). Concomitant segregation of OriC with flanking less-structured macrodomains observed in a recent study (Espeli *et al*, 2008) is consistent with this idea. As *rrn* operons are transcribed coordinately (French & Miller, 1989), it is possible that they are all involved in foci formation. The impaired foci formation in the *hupA/B* mutant on the one hand, and spatial

Fig 3 | Transcription foci localize near OriC at opposite poles of the nucleoid. (A) Fluorescence microscopy image of wild-type cells carrying the *rpoC-yfp* fusion allele and 240 tandem *lac* operator insertions at position 3,908 kb close to OriC (Lau *et al*, 2003). Fluorescence images of RpoC-YFP (RNAP), LacI-CFP (OriC), DRAQ5 (nucleoid) and phase-contrast images are shown in greyscale on the left. Merge images are shown on the right: green is the RpoC-YFP fluorescence signal; red is LacI-CFP; and blue is DRAQ5 fluorescence signal. Scale bar, 2 μ m. (B) Images of wild-type cells carrying the *rpoC-yfp* fusion allele and 240 tandem *lac* operator insertions at position 1,801 kb close to Ter. CFP, cyan fluorescent protein; RNAP, RNA polymerase; Ter, replication terminus; YFP, yellow fluorescent protein.

vicinity of the foci and OriC in the wild-type cells on the other, suggests a loss of the structural–organizational integrity of the *rrn* macrodomain in the *hupA/B* mutant. The corollary is that HU coordinates rRNA production and OriC replication.

The role of HU in stabilizing transcription foci

HU is evenly distributed in the nucleoid and binds equally well to the DNA sites in the OriC and Ter regions *in vivo* (Azam *et al*, 2000; Wery *et al*, 2001; Preobrajenskaya *et al*, 2005; supplementary Fig S5 online), making it difficult to implicate differential binding of HU in generating spatial asymmetry. Similarly, this asymmetry cannot be explained by gene dosage effects or by altered gyrase content in the *hupA/B* mutant (supplementary Figs S1 and S2 online). A previous study proposed the association of HU with RNAP in the nucleoid (Dürrenberger *et al*, 1988), but we do not necessarily infer direct interactions. It is noteworthy that

the supercoils constrained by HU are highly dynamic (Broyles & Pettijohn, 1986). In a co-crystal structure, the HU dimer introduces a positive twist into the DNA (Swinger *et al*, 2003) and an octamer of HU presumably constrains left-handed toroidal coils (Guo & Adhya, 2007), whereas hyperactive HU α can constrain right-handed supercoils (Kar *et al*, 2006). We propose, therefore, that constraint of free DNA supercoils generated during the transcription of *rrn* operons by HU generates metastable structures that maintain the integrity of the foci and any dependent higher-order structures that—by virtue of spatial proximity—might contain OriC. The constraint of DNA supercoils by HU could act as a ‘topological sink’ preventing the diffusion of positive superhelicity and stabilizing the transcription foci (Fig 4B, left side). This metastable structure would thus function as a counting device and, therefore, its extent and also the presence of the foci themselves will depend on continuing transcription. This model is

Fig 4 | Domain organization of the genome. (A) Approximate spatial arrangement of the *rrn* macrodomain with respect to the chromosomal macrodomains determined by Valens *et al* (2004). (B) A model of nucleoid structure organization by HU. At stable RNA operons, the negative twist introduced into the DNA by more than 50 transcribing polymerases (French & Miller, 1989) constrains, in a closed topological domain of about 5 kb in size, a high negative superhelical density (at $-\sigma \sim 0.1$) with compensatory increase in positive superhelicity. Constraint of these positive supercoils (+) by HU acts as a 'topological sink' buffering the diffusion of positive superhelicity and stabilizing transcription (left panel). In the absence of HU, the increased accessibility of gyrase-binding sites in the *rrn* macrodomain imposes an imbalance on supercoil distribution and asymmetry on genomic transcription (right side). NS, non-structured macrodomains; *rrn*, ribosomal RNA; Ter, replication terminus; wt, wild type.

fully consistent with the properties of the hyperactive HU α mutant that not only increases growth rate but also has an enhanced ability to constrain positive supercoils (Kar *et al*, 2005, 2006).

In the absence of HU, the negative supercoils generated during transcription are probably more accessible to DNA-relaxing topoisomerases (Bensaid *et al*, 1996). However, in the *rrn* macrodomain, this relaxation could be counterbalanced both by strong transcription of *rrn* operons and by enrichment of binding sites for gyrase relaxing the positive supercoils. Both these effects would maintain free negative superhelicity available for transcription in the *rrn* macrodomain as opposed to the comparable region comprising Ter, which is devoid of strong *rrn* operons and is also less rich in gyrase-binding sites (Fig 4B, right side). Notably, an asymmetrical distribution of topoisomerase I sites has been implicated in spatial coordination of the replication complex and topoisomerase action in simian virus 40 DNA (Porter & Champoux, 1989).

We propose that perturbation of topological homeostasis due to the loss of HU is compensated by accessibility of DNA-binding sites to gyrase, which not only sustains high negative superhelicity required for optimal rRNA transcription and OriC replication (Crooke *et al*, 1991; Travers & Muskhelishvili, 2005b), but also imposes a spatial pattern on transcription (Jeong *et al*, 2004). Such increased dependency on gyrase explains both the occurrence of gyrase suppressors of HU deficiency and also the hypersensitivity of the HU mutant to gyrase inhibitor novobiocin (Malik *et al*, 1996). We are now designing genetic reporters to test this hypothesis.

METHODS

Strains and plasmids. The wild-type and mutant CSH50 *E. coli* K12 strains were grown in $2 \times$ YT medium at 37 °C, all samples were taken at an absorbance of $A_{600} = 0.4$ –0.6. Genotype and construction details of all strains and plasmids are provided in the supplementary information online.

DNA microarray analysis. Transcript profiling for wild-type and *hupA/B* strains were carried out using *E. coli* DNA microarrays (Ocimum Biosolutions, Hyderabad, India) as described previously (Blot *et al*, 2006). The GStat programme was used to generate statistics on GO terms overrepresented in the transcript profiles (supplementary information online). To visualize the chromosomal distributions of transcript profiles and gyrase-binding sites, positional bins were counted using a sliding window of 200 open reading frames. Two publicly available data sets were used to identify supercoiling sensitive gene transcripts and distributions of gyrase-binding sites (Jeong *et al*, 2004; Blot *et al*, 2006). For details, see the supplementary information online. The ArrayExpress accession number is E-MEXP-1742.

Potassium permanganate assay. The preparation of plasmid topoisomers, high-resolution DNA electrophoresis and determinations of σ were carried out as described by Schneider *et al* (2000). Potassium permanganate reactivity assays *in vitro* and *in vivo* were carried out according to Schneider *et al*, (1999) and Hatoum & Roberts (2008), respectively.

Confocal scanning microscopy. Cells were fixed with formaldehyde as described previously (Cabrera & Jin, 2003), but in 10 mM phosphate buffer (pH 7.4) and incubated on ice for 5 min before being transferred to 18 °C for 1 h. Fixed cells were re-suspended in phosphate buffered saline and fluorescent DNA dye DRAQ5 was added to 50 μ M before mounting the suspension on glass slides. Cells were viewed with a confocal laser scanning microscope (LSM 510 Meta, Carl Zeiss Jena GmbH, Jena, Germany) equipped with argon and helium–neon mixed-gas lasers at excitation wavelengths of 458, 514 or 633 nm. Scans were taken at a resolution of 1,024 pixels $\times$ 1,024 pixels in the line-averaging mode. Image analysis was performed using the LSM 510 software release 3.0 (Carl Zeiss Jena GmbH).

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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