H-NS promotes looped domain formation in the bacterial chromosome

Maarten C. Noom[1], William W. Navarre[2], Taku Oshima[3], Gijs J.L. Wuite[1] and Remus T. Dame[1,4]

The bacterial chromosome is organized into loops, which constitute topologically isolated domains. It is unclear which proteins are responsible for the formation of the topological barriers between domains. The abundant DNA-binding histone-like nucleoid structuring protein (H-NS) is a key player in the organization and compaction of bacterial chromosomes [1,2]. The protein acts by bridging DNA duplexes [3], thus allowing for the formation of DNA loops. Here, genome-wide studies of H-NS binding suggest that this protein is directly involved in the formation or maintenance of topological domain barriers.

Bacterial chromosomes are organized into topological domains that, on average, measure ~10 kb, yielding on the order of 400 such domains per chromosome [4,5]. Topological domains are regions where supercoiling is preserved due to, for instance, attachment to structural components of the cell or DNA duplex cross-linking. The ability of the DNA-binding protein H-NS to bridge DNA duplexes suggested it might serve as a domain barrier [1,3]. Indeed, recently this protein was shown to be involved in topological domain formation *in vivo* [6].

Chromatin immunoprecipitation (ChIP) experiments demonstrate that H-NS binds to A/T rich regions within the genomes of *Salmonella typhimurium* [7,8] and *Escherichia coli* [9,10] where it generally silences transcription. Assuming that adjacent patches of DNA-bound H-NS interact to form a loop, the distance between these patches identified by ChIP corresponds to the size of the resulting loop.

We have determined the location and size of H-NS-bound patches along the genomes of *S. typhimurium* and *E. coli* (Figure 1 and see Figure S1 in Supplemental Data). The patch-spacing distribution follows an exponential decay (Figure 1B) and is thus apparently random. The cumulative probability plot of loop length directly allows comparison with published data (Figure 1C). The average distance measured between patches is ~11 kb in both organisms, in excellent agreement with earlier estimates of topological domain size obtained using other approaches [4,5] (Figure 1C). In stationary phase, when the amount of H-NS is reduced by more than half, the domain size doubles [11]. This correlation between H-NS levels and domain size suggests that H-NS is a dominant factor in setting topological domain boundaries.

There are ~350 H-NS-bound patches along the genome, suggesting that other types of boundaries, for example, due to proteins such as gyrase [12], Fis or TktA [6], may account for the remaining ~15 percent of the 400 topological domains formed during exponential growth [4,5]. The size of a typical H-NS-bound patch is about 2 kb, resulting in 10–15 percent genome coverage in both *S. typhimurium* and *E. coli*. Combined with the average H-NS binding site size of 25 bp [3], this provides an upper limit for the amount of H-NS that is bound to the DNA — 10,000–15,000 dimers. This number agrees well with the estimate of ~10,000 H-NS dimers per cell [1,2], suggesting that most H-NS in the cell is DNA bound. It is believed that chromosome folding preserves the linear order of genes on the DNA; newly replicated segments are condensed right after replication and then move to their final location [13]. If the ordering of the nucleoid is directly coupled to chromosome replication and segregation, a domain is formed when a newly replicated region prone to H-NS binding becomes available. This suggests a model in which the circular chromosome is arranged linearly with H-NS-stabilized loops stacked adjacently.

Previously, it has been suggested that barriers are dynamic and formed stochastically [11]. Our current results refine this interpretation by including sequence determinants (A/T rich regions recognized by H-NS) that are apparently stochastically placed along the genome. The binding of H-NS at defined sites implies an underlying ‘preferred order’ in every cell. Nevertheless, the domains will be dynamic and not continuously fixed at one location in each cell within a population, as domain barriers will differ in stability (with smaller bridged regions being less stable than larger ones). Moreover, the looped domains are reorganized as a result of the effects of growth conditions on H-NS expression levels [1,2], the interplay with other nucleoid-associated proteins [1] and transcription [3,14]. Interestingly, *hns*$^{-}$ strains have a mild phenotype, suggesting that the looped domain organization is largely buffered by the action of other proteins. One such protein might be the H-NS paralogue StpA [15], but even *hns stpA* double mutants do not exhibit a dramatic phenotype in rich medium [15], suggesting that additional proteins serve redundant functions in bacterial chromosome organization (e.g. Fis [16]).

The genomes of *E. coli* and *Salmonella* are highly plastic and a large proportion of their content was obtained by horizontal gene transfer. Horizontally acquired genomic islands are generally A/T rich and thus specifically targeted by H-NS [7–9]. Any newly acquired sequence has the potential to decrease the fitness of its host bacterium by disrupting the organization of the genome. We propose that, in addition to silencing foreign DNA [7], H-NS prevents disruption of the overall nucleoid organization by newly acquired DNA, thereby expanding the role of H-NS as a defense against the potentially harmful effects of foreign DNA.

Our hypothesis that H-NS is a key protein in looped domain formation should be used as a starting point for further experiments in which H-NS-bound patches are explicitly considered as potential domain barriers. It would be useful to

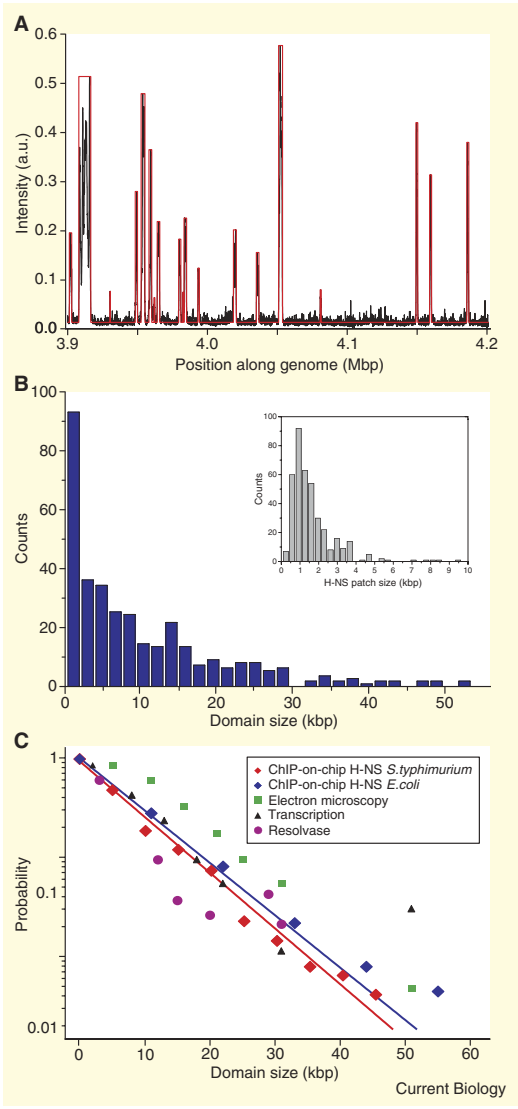

Figure 1. Looped domain formation in the bacterial chromosome.

(A) ChIP-on-chip data for H-NS from *S. typhimurium* [7] (black) and the result of a peak-detecting algorithm applied to these data (red). This algorithm detects peaks with intensity above a threshold, set to three times the background expression level, and a width of more than 240bp. Shown is a 300 kb region representative of the whole genome. Very similar results (not shown) were obtained for *E. coli*. (B) Histogram of interspacing between H-NS patches along the genome. The interspacing between loops was obtained by calculating the distance between two peaks as detected by the algorithm. Inset: Histogram of H-NS patch size. The patch size corresponds directly to the width of the peaks detected. (C) Cumulative probability plot. Plotted along the x-axis is the probability that the size of a domain falls within a certain bin of domain sizes. Accordingly the probability that a domain has a size larger than 0 bp is by definition 1, while that probability for infinitely large loops is zero. Data shown are from H-NS ChIP-on-chip for *S. typhimurium* and *E. coli* (yielding average loop sizes of 10.5 ± 1.0 and 12.3 ± 1.8 kb, respectively, from weighted exponential curve fits of the histogram of interspacing), electron microscopy images, transcription and resolvase assay (published data taken from [4,5]).

systematically repeat the earlier transcription and resolution assays [5,11] on defined regions along the genome. The use of microarrays to study transcription of genes within the proposed loops together with ChIP-on-chip could reveal the presence and dynamics of H-NS-induced domain barriers. The potentially least perturbative approach would be to probe the variance in the physical location of fluorescent tags placed within the proposed loops [13], as this should directly correlate with loop size.

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Supplemental data

Supplemental data (a larger version of Figure 1) are available at http://www.current-biology.com/cgi/content/full/17/21/R913/DC1

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[1]Department of Physics and Astronomy and Laser Centre, Vrije Universiteit, De Boelelaan 1081, 1081 HV, Amsterdam, The Netherlands. [2]Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada. [3]Graduate School of Information Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara, Japan. [4]Department of Physics, Laboratory of Atomic and Solid State Physics, Cornell University, Ithaca, New York 14853, USA. E-mail:rtdame@nat.vu.nl, gwuite@nat.vu.nl