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Bacterial chromatin: converging views at different scales

Remus T Dame[1] and Mariliis Tark-Dame[2]

Bacterial genomes are functionally organized and compactly folded into a structure referred to as bacterial chromatin or the nucleoid. An important role in genome folding is attributed to Nucleoid-Associated Proteins, also referred to as bacterial chromatin proteins. Although a lot of molecular insight in the mechanisms of operation of these proteins has been generated in the test tube, knowledge on genome organization in the cellular context is still lagging behind severely. Here, we discuss important advances in the understanding of three-dimensional genome organization due to the application of Chromosome Conformation Capture and super-resolution microscopy techniques. We focus on bacterial chromatin proteins whose proposed role in genome organization is supported by these approaches. Moreover, we discuss recent insights into the interrelationship between genome organization and genome activity/stability in bacteria.

Addresses
[1] Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, Leiden, The Netherlands
[2] Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

Corresponding authors: Dame, Remus T (rtdame@chem.leidenuniv.nl) and Tark-Dame, Mariliis (m.tark@uva.nl)

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Introduction

Every organism is faced with the challenge of folding of its genome into a confined volume, while maintaining genome activity and imposing functional organization. Bacterial genomes of model organisms such as *Escherichia coli*, *Bacillus subtilis* and *Caulobacter crescentus* are in the order of megabasepairs in size (corresponding to a linear length in the order of millimeters) and they occupy about one third of the volume of the bacterial cell, which is in the order of μm^3. The folding and functional organization of bacterial genomes occurs in a hierarchical manner across different length scales [1,2••,3••,4••]. An important role is attributed to a group of small, abundant, generally basic proteins, which — akin to histone proteins in eukaryotes — act upon genomic DNA to reduce its effective volume, and to generate functional compartmentalization of the genome [5–8]. Generally, these proteins are referred to as Nucleoid-Associated Proteins (NAPs) or bacterial chromatin proteins. Although the architectural properties of many NAPs have been characterized in detail, it has been difficult to investigate their roles *in vivo* due to functional redundancy, as well as the pleiotropic effect of deletion or overexpression of many of these proteins. During the last ten years genome-scale studies of protein binding and gene expression have started to reveal the targets of these proteins, mostly in *E. coli* [9–12]. These binding maps have yielded models of the involvement of these proteins in functional genome organization [13]. Following pioneering studies of eukaryotic genome organization using Chromosome Conformation Capture technology based studies [14–16], three-dimensional information on genome conformation in the bacterium *C. crescentus* became available in 2011 [17]. Since then, several other studies have yielded increasingly detailed knowledge of genome folding in bacteria, including the involvement of specific NAPs [2••,3••,4••]. In parallel, super-resolution microscopy has evolved as powerful tool and has been used to investigate ultrastructural details inside bacterial cells at resolutions in the order of tens of nanometers [18,19]. Over the last few years the application of Chromosome Conformation Capture technologies and super-resolution microscopy has strongly advanced knowledge of genome organization mediated by different NAPs *in vivo*. Here, we discuss the implications of these recent findings, evolving models of bacterial genome organization and directions for future studies.

Global shape of the bacterial nucleoid

Chromosome Conformation Capture technologies provide insight in three-dimensional genome folding by estimation of contact frequencies for genomic sequences in the cell. These technologies rely on chemical cross-linking of the genomic material (i.e. DNA and associated proteins) *in vivo*, followed by genome fragmentation and ligation of cross-linked fragments, often followed by deep-sequencing [14]. Already in the proof-of-principle study of the *C. crescentus* nucleoid [17] several global features of the organization of the nucleoid were detected. The circular genome forms an elongated structure between *ori* attached at one of the poles and *ter* located at the opposite pole. In this configuration interactions between genomically distant loci on the right and left arms occur, reflecting close proximity of the right and left arms extended between the poles. Modeling of

nucleoid structure — using the contact frequencies from the genome-wide interaction map as constraints reflecting physical distance — suggested helical organization with the two arms folded around each other similar to the fluorescence microscopy observations in other bacteria (see below). A more recent study of the *C. crescentus* nucleoid achieved much higher resolution [2••]. It confirmed the helical organization. In addition more than 20 Chromosomal Interaction Domains (CID's), regions of the genome within which loci interact more frequently with each other than with loci in other domains, were identified. CID's ranged in length from 30 to 420 kb. These structures and their boundaries are re-established following replication. Boundaries of CID's seem to correlate with expression of highly expressed genes. Inhibition of transcription using rifampicin, a drug with potential pleiotropic effects, disrupts CID boundaries. Also moving of a locus with highly expressed genes into a poorly expressed region of the genome, results in the generation of a new CID boundary. Additional studies are needed to unambiguously determine the nature of CID boundaries. The binding of NAPs might also be involved in generating boundaries. Two proposed architectural components of the nucleoid (HU and SMC) were explicitly investigated, but were found not to be involved in boundary formation. Instead, HU facilitates genome compaction *in vivo*, by promoting short-range contacts along the genome, confirming a microscopy study of local genome conformation [20] and *in vitro* properties of the protein [21,22]. Absence of SMC, which is known to form ring-like structures around DNA [23], potentially acting as a bridge between two DNA duplexes reduces the frequency of interactions between the arms, while increasing the range of loci exhibiting interactions. This suggests that in *C. crescentus* SMC does not compact DNA, but rather is important in aligning the chromosome arms, by mediating arm-arm contacts.

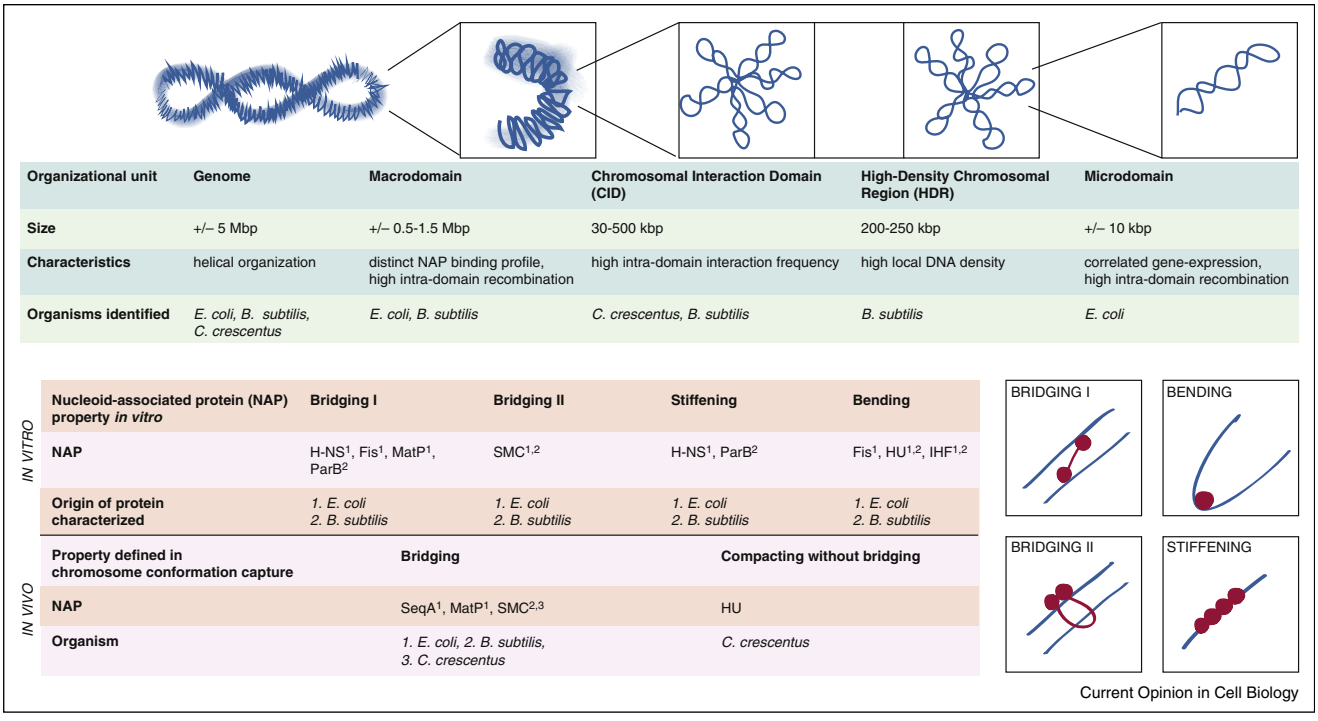
Two very recent independent high-resolution Chromosome Conformation Capture studies on the *B. subtilis* nucleoid reach similar conclusions in relation to global organization of the genome [3••,4••]. The *B. subtilis* nucleoid exhibits interactions among loci along and between the two chromosome arms, corresponding with an overall chromosomal configuration in which the arms are extended between *ori* and *ter* at opposite ends of the cell. Also, CID's as observed in *C. crescentus*, ranging in length from 50 to 300 kb are detected in both studies [3••,4••]. Although 60% of the CID boundaries also correlate with highly transcribed genes [3••,4••], other barriers (30%) correlate with regions of higher than average AT-content, bound by the NAP Rok [3,24]. The large interaction domains seen in recent Hi-C studies could point at organization of the *B. subtilis* genome in large macrodomains as described over a decade ago in *E. coli* [25]): the *ori* domain encompasses a region of ~1.5 Mbp, the smaller *ter* domain is ~500 kbp in length and located centrally along the arms the *left* and *right* macrodomains exhibit close juxtaposition [3••]. In *E. coli* macrodomains have been operationally defined as regions with high internal frequency of recombination [26]; the nature of macrodomain boundaries in *E. coli* is not known. A crucial role in genome organization is attributed to ParB, which according to the prevailing model nucleates binding at *parS* sites, and forms extended filaments along DNA, possibly involving DNA bridging [27–29]. An alternative model explains ParB spreading around *parS* by caging, obviating the need for filament formation or DNA bridging [30]. ParB binding is required for SMC recruitment [31,32] and alignment of the two chromosomal arms [3••,4••]. Both studies further dissect the internal organization of the *ori* domain, revealing the existence of hairpin-like folds within this domain [3••,4••], mediated or stabilized by ParB and SMC, which were experimentally mapped within the folded domain by Chromatin Immuno Precipitation (ChIP) [3••]. Chromosome Conformation Capture studies have also been carried out on fast growing *E. coli* cells and cells with artificially induced starvation [33]. Whereas these studies detect the *ori* and *ter* domains, no further internal organization — as seen for the other investigated species — is captured. It is to date unclear whether this is due to a fundamental difference in organization, related to the fact that the cells are not synchronized or — in part — a consequence of the high-salt nucleoid isolation procedure that yields swollen nucleoids, attributed to dissociation of DNA binding proteins [34]. SeqA and MatP, two non-classical NAP's associated with the *ori* and *ter* domains [1], respectively, mediate interactions *in trans* that shape these domains [33].

Using conventional fluorescence microscopy it is hard to resolve much detail in the bacterial nucleoid due to its small size and due to limited resolution. It was suggested a decade ago based on deconvolved fluorescence microscopy images that nucleoids stained by incorporated fluorescent nucleotides in *B. subtilis* are helically organized [35]. Similar global structure was independently identified in Chromosome Conformation Capture studies [3••,4••]. Helical organization was also observed in similar microscopy studies of nucleoids in *E. coli* [36,37•], stained using NAPs fused to fluorescent proteins (FPs). Super-resolution microscopy allows visualization of features much smaller than the nucleoid [18,19], detection of heterogeneity in nucleoid density and spatial protein-distributions. In a pioneering study in *E. coli* the location of different NAPs fused to photoactivatable FPs (PA-FPs) was determined. The majority of NAPs investigated (HU, IHF, FIS and StpA) are found scattered throughout the nucleoid [38]. HU-PA-FPs exhibit a similar scattered distribution in *C. crescentus* [39]. The H-NS-PA-FP fusion investigated in *E. coli* behaved different from the other NAP-PA-FPs studied and was found to localize in two dense clusters per genome equivalent [38]. This behavior of the H-NS-PA-FP fusion — although surprising — was

in agreement with the proposed ability of H-NS to bridge genomic DNA segments *in vivo* [13,40,41] (see below). In a follow-up article from the same laboratory the strong clustering observed earlier was considered an artifact attributed to aggregation of the used PA-FP [42]. Finally, a super-resolution study of the nucleoid in *B. subtilis*, using HU-FP fusions or direct DNA staining using an intercalating dye, confirmed folding of the nucleoid into a helicoidal shape, and revealed nucleoid domains of higher density, referred to as High-Density Chromosomal Regions (HDR's) [3••]. About 10 HDR's are present per genome equivalent independent of the cell cycle stage; the amount of genomic DNA condensed within these regions is estimated at 200–250 kbp.

The studies discussed above are the first to provide indications of how genomes are organized at the global scale and of which cellular processes/factors have a role in determining this structure. However, much remains to be revealed in terms of changes in genome structure upon growth in different environmental conditions such as different nutrient availability, osmolarity, temperature, oxygen availability, all known to lead to changes in global gene expression patterns. Earlier studies of NAPs evolved at the scale of individual proteins operating in shaping the genome. Many NAPs — primarily from *E. coli* — have been investigated *in vitro* in relation to their roles in DNA organization; specific architectural properties have been attributed to most of these proteins. DNA bending properties have been attributed to proteins such as HU, IHF and FIS based on *in vitro* studies, suggesting generic roles for these proteins in compacting DNA [1,5,6,43]. Such a generic role is supported by the scattered localization of these proteins throughout the nucleoid as discussed above. In addition, IHF and FIS bind sequence specifically, often directly upstream of promoter regions, and play specific roles in transcription regulation [44,45]. Other proteins, such as H-NS-like proteins and SMC have been shown *in vitro* to be able to bridge DNA duplexes [8,46–48] and in the case of H-NS-like proteins, to form filaments along DNA [47]. H-NS like proteins have been implied in gene repression, attributed to their ability to 'spread' along DNA [47] covering intergenic regions or intragenic regions, supported by genome-wide ChIP and transcription studies [9–11,49]. Other studies comparing the effects of the two types of H-NS-DNA complexes on RNA polymerase progression reveal an inhibitory effect of bridged regions only [50••]. It remains

Figure 1



Organizational unit	Genome	Macrodomain	Chromosomal Interaction Domain (CID)	High-Density Chromosomal Region (HDR)	Microdomain
Size	+/– 5 Mbp	+/– 0.5-1.5 Mbp	30-500 kbp	200-250 kbp	+/– 10 kbp
Characteristics	helical organization	distinct NAP binding profile, high intra-domain recombination	high intra-domain interaction frequency	high local DNA density	correlated gene-expression, high intra-domain recombination
Organisms identified	*E. coli, B. subtilis, C. crescentus*	*E. coli, B. subtilis*	*C. crescentus, B. subtilis*	*B. subtilis*	*E. coli*

IN VITRO

Nucleoid-associated protein (NAP) property *in vitro*	Bridging I	Bridging II	Stiffening	Bending
NAP	H-NS[1], Fis[1], MatP[1], ParB[2]	SMC[1,2]	H-NS[1], ParB[2]	Fis[1], HU[1,2], IHF[1,2]
Origin of protein characterized	*1. E. coli 2. B. subtilis*	*1. E. coli 2. B. subtilis*	*1. E. coli 2. B. subtilis*	*1. E. coli 2. B. subtilis*

IN VIVO

Property defined in chromosome conformation capture	Bridging	Compacting without bridging
NAP	SeqA[1], MatP[1], SMC[2,3]	HU
Organism	*1. E. coli, 2. B. subtilis, 3. C. crescentus*	*C. crescentus*

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Multi-level organization of bacterial chromatin across different species. The genome is folded in a helical elongated shape along the long cell-axis and divided into macrodomains. In addition, throughout the genome organization occurs at a lower scale in Chromosomal Interaction Domains and High-Density Chromosomal Regions. Finally, at the lowest scale organization occurs in microdomains. Nucleoid-associated proteins contribute to genome-organization via their effects on DNA structure and conformation. Tables summarize the characteristics related to different types of organization, the organisms in which this type of organization has been observed and in case of NAPs indicates whether characteristics have been identified *in vitro* and/or *in vivo*. Note that Chromosome Conformation Capture does not reveal mechanistic details in relation to the exact mode of binding/architectural properties of NAPs.

to be defined what type of structures formed by H-NS are relevant *in vivo*.

H-NS has been implied in the stabilization of genomic loops, topologically isolated microdomains ~10 kb in size [51,52], way below the size of CIDs identified in *C. crescentus* and *B. subtilis* (see above). The distribution pattern of H-NS-bound regions along the genome is in support of a model in which H-NS mediates the formation of loops [13]. The observation that the barriers of microdomains are stochastically positioned [52–54] suggests that establishing H-NS mediated loops is stochastic involving different genomic regions at different moments in time or cell-to-cell variation. A model of the hierarchical organization of bacterial genomes is given in Figure 1.

Outlook

The bacterial nucleoid is organized at different length scales, details of which have been now investigated using various *in vivo*, *in vitro* and *in silico* approaches. The field has now reached a point at which this combined knowledge starts to be integrated in unifying models, which can be validated.

Currently the main challenge is to disentangle the relationship between genome organization and transcription, specifically to determine whether and how structural properties of NAPs as observed *in vitro* translate into genome organization *in vivo*. Chromosome conformation capture studies available reveal some correlation between observed structure and NAP binding [2••,3••,4••]. It is long known that nucleoid structure and global transcription patterns are altered as a function of environmental conditions. [55,56] These changes are — in part — attributed to altered NAP expression levels and the interplay between NAPs [6,43,57]. A true understanding of the involvement of NAPs requires determination of correlations between global and local genome structure and NAP binding under changing bacterial growth conditions, as well as of transcription.

It is tempting to propose generic models of NAP function in genome organization based on the currently available *in vitro* and *in vivo* data. However, it should be kept in mind that detailed *in vivo* chromosome capture maps are available for *C. crescentus* and *B. subtilis*, while *in vitro* studies of NAPs from these species is largely unavailable. On the other hand, NAPs of *E. coli* have been thoroughly characterized, but detailed insight in *in vivo* conformation is lacking. It is not obvious that genome organization is the same in different bacteria. Therefore inter-species extrapolations regarding different levels of nucleoid organizations should be made with care.

It is evident that chromatin structure affects transcription, and potentially also replication, DNA repair and mutagenesis. The number of *in vivo* studies on interplay between chromatin and such DNA transactions is small and often inconclusive as they are complicated by the pleiotropic roles and partial functional redundancy of NAPs [58–60]. With the emergence of genome-wide binding-maps of NAP proteins and intra-chromosomal contact maps, we expect that also the interplay between these other DNA-based transactions, possibly associated with genome evolution and nucleoid structure will be unravelled.

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