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The organization of bacterial genomes: Towards understanding the interplay between structure and function

Daan J. W. Brocken[1], Mariliis Tark-Dame[2] and Remus T. Dame[1,3]

Abstract

Genomes are arranged in a confined space in the cell, the nucleoid or nucleus. This arrangement is hierarchical and dynamic, and follows DNA/chromatin-based transactions or environmental conditions. Describing the interplay between local genome structure and gene activity is a long-standing quest in biology. Here, we focus on systematic studies correlating bacterial genome folding and function. Parallels on organizational similarities with eukaryotes are drawn. The biological relevance of hierarchical units in bacterial genome folding and the causal relationship between genome folding and its activity is unclear. We discuss recent quantitative approaches to tackle these questions. Moreover, we sketch a perspective of experiments necessary to iteratively and systematically build, test and improve structure–function models of bacterial chromatin.

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

[3] Centre for Microbial Cell Biology, Leiden University, Leiden, The Netherlands

Corresponding author: Dame, Remus T. (rtdame@chem.leidenuniv.nl)

Email address: d.j.w.brocken@lic.leidenuniv.nl (D.J.W. Brocken), m.tark@uva.nl (M. Tark-Dame)

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Introduction

Genomes of all organisms, bacteria, archaea and eukaryotes, are arranged in the cell in a confined space, the nucleoid or nucleus. This arrangement is dynamic allowing for DNA transactions such as replication, transcription and repair to occur at appropriate times. A spectrum of mechanisms is involved in physically compacting and functionally organizing genomes in cells. Although at first sight the organization of the genomes of bacteria and eukaryotes may appear diverse, common principles are recognized [1]. The proteins providing structural and functional organization are generally not conserved at the protein sequence level. Nevertheless, several types of conserved structural features are evident [1]. Bacterial H-NS-family proteins, SMC proteins, and eukaryotic insulator proteins bridge DNA to form loops at different length scales [2–4]. In bacteria DNA decorated with architectural proteins is folded in looped structures [5], whereas in eukaryotes nucleosomal fibres, in which DNA is wrapped around histone proteins, are arranged into loops [6]. Although in eukaryotes much of genome regulation occurs at the level of nucleosomes (via histone tail modifications and nucleosome density) [7,8], at a coarse grained, structural level such molecular details are irrelevant. At a larger scale both in bacteria and eukaryotes, loops are arranged into structural domains, defined by genome activity [1,9]. An understanding of the interplay between structural and functional organization is emerging. The field is further advanced in eukaryotic organisms compared to bacteria, yet in both cases a lot of unanswered questions remain. Here, we discuss recent advancements in understanding bacterial genome organization, linking chromatin structure to function. Our focus is on systematic approaches aimed at determining characteristics of the dynamic organization of bacterial genomes, and lessons learned from similar studies in eukaryotic model systems.

State of the art

Most bacterial model organisms harbour a single circular chromosome. The bacterial chromosome has been primarily studied in *Bacillus subtilis*, *Escherichia coli* and *Caulobacter crescentus*, and unless otherwise indicated the information summarized here applies to these organisms. Bacteria have a cell cycle with a duration on the order of tens of minutes. As a consequence, genome folding and transcription are intimately coupled with genome replication. Current key question is to understand the structure–function relations within the bacterial chromosome, specifically the interplay between genome structure and gene activity.

The first systematic studies of bacterial chromosome structure aimed at defining the positioning of genomic loci within the cell. Two approaches based on fluorescence microscopy were used: *i*) fluorescent *in situ* hybridization (FISH) labelling of endogenous loci in fixed cells [10] and *ii*) fluorescent repressor-operator systems (FROS) involving binding of e.g. LacI-GFP to exogenous *lacO* operator sites integrated in the genome in living cells [11,12]. These studies have revealed that regions proximal to the initiation (*oriC*) and termination (*ter*) site of replication are not distributed randomly in the nucleoid but exhibit specific localization patterns throughout the cell cycle [12–14]. Visualizing the locations of up to about 100 defined genomic loci relative to *oriC* reveals a linear relationship between genomic and physical location, indicating a linear ordering [15–17]. The reproducible positioning of genomic loci at specific subcellular positions in individual cells and their linear organization appear as fundamental features of chromatin organization in bacteria.

In *E. coli*, *oriC* and *ter* are part of two distinct structural domains, the Ori and Ter macrodomains [18]. In addition, the *E. coli* genome contains two other structural domains flanking the Ter domain, called the Right and Left macrodomains, and two non-structured (NS) regions, flanking the Ori domain [19,20] (see Figure 1). The Ter domain stretches along the length of the cell to connect the two chromosomal arms, with an estimated packing density of only 1/10 compared to the rest of the genome [16]. Genome packing density may correlate with genome activity. The chromosome is organized as a dense nucleoid scaffold wherefrom large 'plectonemic' loops of negatively supercoiled DNA protrude. Such loops are probably formed by binding of a group of proteins called 'nucleoid-associated proteins' (NAPs) [21–23]. But these proteins only provide part of the answer.

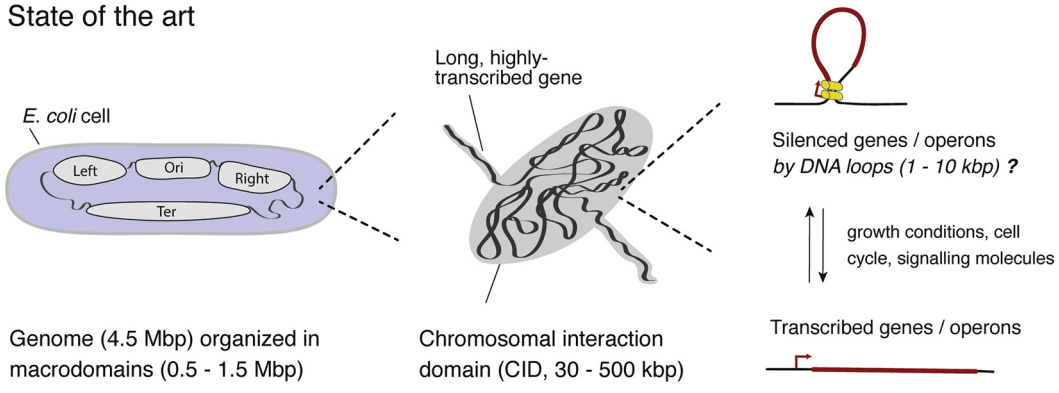
What is the relevance and evolutionary conservation of the different levels of organization? How are the different organizational units and their borders defined, and what are their dynamics upon DNA transactions? Below we discuss recent advances on quantitative approaches aiming to find answers to these fundamental questions.

Chromatin structure: organization in domains

Since the early 2000's chromosome conformation capture (3C), developed by Dekker and colleagues [24], and derivatives of the method, have promoted large advances in understanding genome folding and function [25–28]. These techniques yield maps of relative interaction frequency between different pairs of genomic sites averaged over a population of cells. The technique relies on chemical cross-linking, digestion, religation of digested fragments, followed by identification of the hybrid DNA molecules [24]. A large-scale variant, Hi-C [29], has been used to produce genome-wide contact matrices in several organisms. These matrices, structurally interpreted by modelling approaches, provide insight in global and local features of genome structures [28,30].

Among bacterial species, currently, Hi-C contact maps are available for *C. crescentus* [31–33], *B. subtilis* [34–36] and *Mycoplasma pneumoniae* [37]. Different genome features have been identified in these studies. In the *C. crescentus*, *B. subtilis*, *V. cholera and M. pneumoniae* genome-wide contact maps, several tens of chromosomal interaction domains (CIDs) have been identified [31,34,35,37,38] (see Figure 1). CIDs are highly self-interacting genomic regions. These regions vary in size from about 20 to 400 kbp, and they are analogous to so-called topologically associated domains (TADs) identified in contact maps of eukaryotic genomes [39,40]. An

Figure 1



State of the art. The bacterial genome is organised at different length scales. At the smallest length scales it has been hypothesised that genome folding is directly affected by environmental signals which are translated into a transcriptional response.

exception in terms of CID dimensions (15–33 kbp) is the genome of *M. pneumoniae*, which is only 800 kbp in size [37], 5x smaller than the genomes of other bacteria studied by Hi-C. For *E. coli*, a genome wide contact map was obtained using an other 3C-derivative [41]. Whereas regions of high internal interaction are identified, the relatively low resolution of these maps does not permit identification of CIDs. The regions of high interaction frequency might correspond to the macrodomains discussed above. Indications of the existence of domains on average ~10 kbp in size (microdomains) in *E. coli*, comparable in size to CIDs, come from EM imaging of isolated chromosomes, clustered gene activity [42] and *in vivo* recombination based assays in the closely related *Salmonella typhimurium* [43,44]. Finally, at a length scale between that of CIDs/microdomains and macrodomains, another organizational structure is proposed: the high-density chromosomal regions (HDRs), of which 10 per genome are estimated and sizing around 200 tot 250 kbp [35].

Considering the mechanisms that form CIDs and their boundaries, mounting evidence is pointing at long (>1 kbp), highly transcribed genes separating domains [31,32,34,35,38]. Inhibition of transcription with rifampicin almost completely eliminated these borders [31,34,36], and insertion of a highly expressed gene was sufficient to generate a new barrier [31]. Possibly, these highly transcribed genes cluster into transcription factories [45], imposing an organization of intervening sequences in looped domains. Borders not containing highly expressed genes often have low GC%, indicating the presence of horizontally acquired elements [31,35]. In that light it is interesting to note that low GC-content regions are targeted by the nucleoid-associated proteins H-NS and FIS [46–48]. Inhibition of gyrase reduces the sharpness and position of CID borders, which has been interpreted as supercoiling being important in establishing CIDs [31,37]. Thus, CIDs have been proposed to be connected by segments of decompacted chromatin, forming a higher-order "domains-on-a-string" organization. The biological relevance of this level of organisation is not clear.

CIDs and their borders are dynamic and correlate with changes in gene expression. It has been long known that bacteria change global gene expression patterns in response to environmental cues [49]. Indeed, contact maps acquired from bacterial cultures in different growth conditions, e.g. starved cells versus exponentially growing cells, exhibit clear alterations in CID boundary positioning [32]. Currently, there is not sufficient data to correlate the changes occurring in global genome folding (at CID level or higher) and changes in the expression of specific genes and operons in response to altered conditions.

Structure–function relations of the bacterial genome

Correlations, linking genome structure to gene activity, have been established by combining information from chromosome conformation capture, chromatin immunoprecipitation (ChIP) data of DNA-binding proteins and gene expression profiles. In eukaryotes insulator proteins are bound at domain boundaries and have been shown to be involved in boundary formation [6,39,50,51]. There is no direct evidence for the involvement of specific architectural proteins in CID boundary formation in bacteria, but for microdomains in *E. coli*, which might be the same as CIDs, the involvement of nucleoid-associated proteins FIS and H-NS has been suggested [52,53]. There is very limited information on structure–function relations, but more is known about the effect of DNA-binding of NAPs on gene expression. Genes bound by H-NS are generally expressed at low levels or silenced completely [47,48,54,55]. Such an effect on gene expression is not seen for binding of FIS, which primarily exerts effects on transcription indirectly by regulating the expression of other transcription factors [47,56]. High protein occupancy (including other DNA-binding proteins in addition to NAPs) along the genome is also associated with gene silencing [57].

Interplay between DNA-binding of NAPs and gene expression cannot provide us with an understanding of genome structure-driven regulation of gene activity (see Figure 1). To understand how local genome organization at the level of genes and operons affects functional biological outcome, e.g. state and level of gene expression, studies on single cells or pre-sorted small homogenous cell populations are needed. For instance, chromosome conformation capture studies of genome organization involve ensemble-averaging over the genomic conformation of all cells in a sample. This is a particularly important limitation for using this technique in bacteria with short cell cycles and/or which are hard to synchronize. The benefit of single-cell methods is that in contrast to yielding average characteristics of the population of cells analysed, they reveal intercellular variance within a population, allowing identification of differently behaving subpopulations of cells [58]. The solution to avoid ensemble-averaging in Hi-C is to use single cells as shown for eukaryotes [58,59].

3C techniques cannot be used to straightforwardly determine changes in genome conformation occurring at short time-scales, such as regulatory switches in response to environmental cues, due to limited time resolution. To quantify dynamic changes in chromatin structure at the scale of genes and operons (on the order of 1–10 kilobases), a different approach is required. Currently, high-resolution imaging of sets of *in vivo* fluorescently tagged loci encompassing the genomic

region of interest in living cells, e.g. using FROS, is best suited to answer these questions. Performing time-course experiments on these single cells and/or molecules allows for characterization of the true dynamics of genome organization and gene activity regulation. Positioning of loci relative to each other and cellular landmarks can be determined in real-time to reveal changes occurring upon varying growth conditions. Hensel and co-workers demonstrated the feasibility of such methods in bacteria: they investigated the formation of loops of 2.3 kb upon binding of the cI repressor using two FROS arrays flanking the operator elements that bind cI [60]. A positive correlation was established between loop formation due to repressor binding and gene activity (simultaneous positive autoregulation and silencing of a major lytic promoter). This approach can be extended to studies correlating local genome organization to gene activity.

Additionally, tracking of loci in living cells allows determination of diffusion constants (i.e. the space explored per time unit by a locus). These values vary as a function of growth phase and are subject to metabolic processes, ATP synthesis and temperature [61]. Moreover, these values differ dependent on subcellular localization and chromosomal coordinate [62–64]. Loci in the Ter macrodomain are least mobile; mobility increases along the chromosomal arms towards the Ori macrodomain [62]. It is not clear whether gene activity also correlates with its macrodomain-positioning, but there are indications that active genes in the Ori macrodomain are higher expressed than those in Ter. The mechanistic nature of these differences remains unclear, but cannot be simply attributed to a gene dosage effect [65]. Gene silencing does not correlate with macrodomain positioning, but with regions of high levels of nucleoid-associated protein binding [65,57,66], which might be more compactly organized compared to regions with active transcription. Systematic parallel studies of gene activity and global and local genome organization are needed to establish firm correlations.

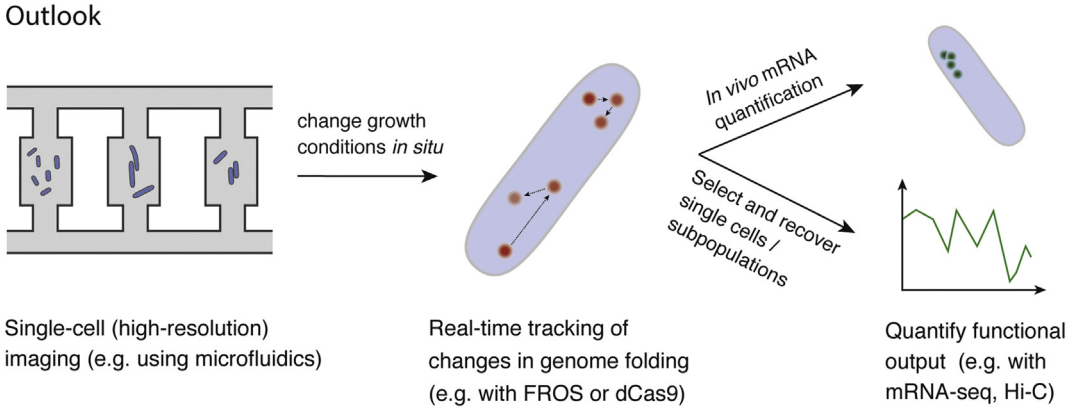
Outlook

FROS and Hi-C have advanced our knowledge of genome organization *in vivo*. Questions of structure–function relations can be addressed by either of these methods, but particularly powerful will be their mutual combination or combination with other genomic analyses (e.g. RNA-seq, ChIP-seq). We see three promising avenues for future research (see Figure 2):

1) application of the programmable and DNA-targeting platform CRISPR/dCas9, which is replacing FROS in many recent studies in eukaryotes [67–70], in bacteria. Advantage is that this approach obviates extensive time-consuming genome engineering,
2) establishing Hi-C for low number of cells permitting analyses of homogenous sub-populations extracted from intrinsically heterogeneous bacterial populations,
3) developing parallel imaging platforms for single bacterial cells under controlled variable conditions. This is essential for obtaining systematic data from time course studies. Clever microfluidic channel designs – some of which already utilized now – can be used to change growth conditions leading to a physiological response whilst cells are being imaged [71–73]. Application of microfluidics might be the key to recover (defined populations of) single cells, which can be processed for Hi-C or genomic analysis methods.

Instrumental to all these approaches is the verification of function i.e. quantification of the level of gene activity, which can be measured by (single-cell) mRNA-seq [74,75] or visualization of real-time kinetics of transcription *in vivo*. Transcription *in vivo* can be

Figure 2



Outlook. Development of new technologies and application of existing – but in bacteria unused – technologies is expected to establish structure–function relationships for bacterial genome organisation.

visualized e.g. by including RNA aptamers in mRNA transcripts targeted by MS2-GFP [76,77], the RNA-binding protein Pumilio [78,79] and the nuclease-deficient Cas9 (dCas9) from type II CRISPR/Cas systems [80], which can be (re-)programmed to specifically bind RNA [81].

It is remarkable that very few studies to quantitatively dissect structure–function relations on local scales have been published, whereas the techniques required are either already implemented and applied, or just need translation from eukaryotic to prokaryotic cells. We expect extensive data to become available in the next few years that can be used for iterative building, testing and improving of biological models of genome organization and dynamics.

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- •• of outstanding interest

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