

Physical descriptions of the bacterial nucleoid at large scales, and their biological implications

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Abstract

Recent experimental and theoretical approaches have attempted to quantify the physical organization (compaction and geometry) of the bacterial chromosome with its complement of proteins (the nucleoid). The genomic DNA exists in a complex and dynamic protein-rich state, which is highly organized at various length scales. This has implications for modulating (when not directly enabling) the core biological processes of replication, transcription and segregation. We overview the progress in this area, driven in the last few years by new scientific ideas and new interdisciplinary experimental techniques, ranging from high space- and time-resolution microscopy to high-throughput genomics employing sequencing to map different aspects of the nucleoid-related interactome. The aim of this review is to present the wide spectrum of experimental and theoretical findings coherently, from a physics viewpoint. In particular, we highlight the role that statistical and soft condensed matter physics play in describing this system of fundamental biological importance, specifically reviewing classic and more modern tools from the theory of polymers. We also discuss some attempts toward unifying interpretations of the current results, pointing to possible directions for future investigation.

(Some figures may appear in colour only in the online journal)

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1. Introduction

The demarcation line between a ‘physical’ and a ‘biological’ system is rapidly becoming anachronistic, to the point that it possibly hinders research in both disciplines. This is particularly true in the context of gene expression, which is a fundamental process in biology at the molecular level, common to all life on earth: the genetic code is read out (‘transcribed’) from DNA and written into RNA, which, in the case of messenger RNA, is then translated into proteins. In order for the right number of proteins to be produced in response to changes in environment, internal states, and stimuli, all cells are capable of tightly regulating this sequence of events (Alberts *et al* 2008). The physico-chemical implementation of this gene regulation process takes place primarily through specific DNA-binding interactions of transcription factors, which repress or promote transcription.

It is becoming very clear that the genome’s conformational properties as a polymer come into play in the processes involved in the regulatory fine-tuning of gene expression, in particular its topological, chemical, geometric and mechanical properties. These properties can influence the activity of the transcription factors and can play a role in the coordination of a large-scale cellular response. Evidence for this level of regulation has been found in the different kingdoms of life. We focus here on the efforts to describe the genome’s physical state in the case of bacteria, where it is (perhaps surprisingly) less explored than for higher life forms.

Understanding this problem requires stringent, quantitative experiments with the standards of physics, together with up-to-date physical models and arguments. Since considerable knowledge has been developed in polymer science over the last 50 years, it is very tempting to try to apply this knowledge to understand the energy and time scales involved in maintaining the DNA compact, and at the same time accessible inside a cell, and the role of its geometry, structure and compaction in gene regulation.

This review discusses challenges that arise in the biological arena, for progress in which mature experimental and theoretical tools from the physical sciences might now allow significant progress. The review is primarily aimed at our colleagues in the physical sciences: it should communicate a feel for the main questions and the main challenges, and our understanding that a comprehensive physical approach is possible and necessary at this point. We will not explain the physical models in great technical detail, and we hope this work will also be of interest to biologists who could take the references given here as a starting point and a ‘compass’ in order to evaluate different modeling approaches. Ultimately, we believe that optimal progress in this area will take place in collaboration, and this review might contribute to establishing a common ground and language.

2. Background

Bacteria are single cell organisms of fundamental importance in nature and to mankind. They are ‘prokaryotes’, in the etymological sense that they lack a nucleus as a compartment

Box 1. Main factors affecting nucleoid organization.

Supercoiling by topoisomerases. These enzymes affect the winding of DNA, and play a role in the creation of the observed branched structure of plectonemic loops along the genome. *Nucleoid-associated proteins (NAPs).* These proteins bind to DNA with different specific modes, each responsible for a different aspect of organization, ranging from double-strand bridging to nucleoprotein filament formation. *Confinement.* Millimeters of genomic DNA are confined within the small cell volume of a bacterium. *Molecular crowding.* There is a high concentration of macromolecules present in the cytoplasm. This factor could affect nucleoid organization at different levels, for example creating a general effective self-attraction favoring collapse, and strong depletion attraction between large objects, such as ribosomes. *Replication and transcription.* These are the non-equilibrium processes of DNA and mRNA production that continuously take place in dividing cells. The first affects the sheer amount of genome present and supercoiling and makes both highly non-steady, the second can create uneven ribosome concentrations. Both contribute to non-thermal force and displacement fluctuations of the chromosome.

enclosed by a membrane (in contrast to the ‘eukaryotic’ cells that make up animals and plants). Indeed, all the DNA, RNA and proteins in the bacterial cell are always present together in the same single compartment. While the classical picture of bacteria (still implicitly adopted by many theoretical and experimental investigators) views them as little more than a bag (or ‘well-stirred reactor’) of proteins and DNA, it is now clear that this tenet is flawed on many different levels. Despite their lack of membrane-bound organelles, bacteria have a high degree of intracellular spatial organization, related to most cellular processes. Perhaps the highest organized structure of the bacterial cell is the genome itself.

In most bacteria, the chromosome is a single circular DNA molecule confined by the cell membrane. In *E. coli* (the best studied bacterial system), the chromosome consists of about 4.7 million base pairs (bp) and has a total length of 1.5 mm (Stavans and Oppenheim 2006, Trun and Marko 1998). Bacterial DNA is organized into a specific structure called the nucleoid, which is composed of DNA, RNA and proteins, and occupies a well-defined region of the cell (Sherratt 2003). The nucleoid is organized by a set of nucleoid-associated proteins, or ‘NAPs’ (such as Dps and transcription factors Fis, H-NS, IHF, HU), which can modify the shape of the DNA both at local and global levels (Luijsterburg *et al* 2006, Ohniwa *et al* 2011). Since the linear size of the genomic DNA is orders of magnitude larger than the length of the cell, it must be packaged and organized in such a way that the resulting structure is compact, while still allowing the primary information processing, genome replication and gene expression (Thanbichler *et al* 2005). In fact, recent evidence strongly suggests that changes in chromosome architecture can directly affect the accessibility and activity of the regulatory proteins at the local level as well as at larger scales. In addition, the genome can efficiently control gene expression by changing the way DNA-binding regulatory proteins can access their target sites via the chromosome architecture (Dillon and Dorman 2010). Historically, the nucleoid has been visualized by transmission

electron microscopy (TEM), phase-contrast microscopy and confocal scanning light microscopy; an overview of early microscopic visualization of the nucleoid can be found in Robinow and Kellenberger (1994). These studies showed that the nucleoid mostly occupies a separate subcellular region, without being bound by a membrane, and that thin DNA threads extrude from this region.

The double-stranded genomic DNA is generally torsionally constrained in bacteria, typically in such a way that its linking number (the number of times each single strand of DNA winds around the other) is lower than in the relaxed configuration. In biological words this is referred to as ‘negatively supercoiled’, and the specific difference in linking number is called superhelical density. The name is due to the fact that supercoiled DNA develops non-zero writhe, or ‘supercoils’, i.e. it is wrapped around itself in the manner of a twisted telephone cord. This torsional constraint has important consequences for gene expression, as the mechanical stress carried by a negatively supercoiled configuration can locally weaken the interaction between the two strands. The resultant breaking of base-pair bonds between the two helices is required for the initial steps of transcription, as well as DNA replication and recombination. Since most DNA-binding proteins bind to DNA sensitively to the arrangement of the two strands, and in particular to their average distance, negative supercoiling usually also facilitates protein binding (Dillon and Dorman 2010). The level of supercoiling is tightly regulated by the cell, and it can be changed by the action of specific enzymes such as topoisomerases and gyrases.

The average supercoiling is generally negative in bacterial cells (except for thermophilic bacteria (Confalonieri *et al* 1993)). In the case of *E. coli*, the supercoiling superhelical density is maintained around the value $\sigma = -0.025$, where the supercoil density σ is defined as the relative change in linking number due to the winding (or unwinding) of the double helix, where negative values of σ correspond to an unwound helix (Stavans and Oppenheim 2006). This value is set by the constraining action of DNA-binding proteins and the combined activity of topoisomerases. Deviations larger than 20% to either side of σ are detrimental to cell growth. For example, overwinding causes the formation of DNA structures that impede transcription and replication, while excessive underwinding leads to poor chromosome segregation (Stavans and Oppenheim 2006).

The most important *features linking nucleoid organization and cell physiology* are summarized here, and discussed further in the review. See also box 1 for a brief description of the main factors affecting nucleoid organization.

(i) At large scales, it is seen from *in vitro* experiments that the nucleoid is composed of topologically unlinked dynamic domain structures; these are due to supercoiling forming plectonemes and toroids (Marko and Cocco 2003), and stabilized by NAPs, for example Fis. This combination of effects gives the chromosome the shape of a branched tree-like polymer visible from TEM (Kavenoff and Bowen 1976, Postow *et al* 2004, Skoko *et al* 2006). Topological domains are thought to be packaged during replication, otherwise it appears that their boundaries are ‘fluid’ and

randomly distributed (Postow *et al* 2004, Thanbichler and Shapiro 2006).

- (ii) Strong compaction is experimentally observed *in vivo*, and possibly arises from confinement within the cell boundaries, but also from various factors such as molecular crowding (de Vries 2010) and supercoiling (Stuger *et al* 2002). The degree of compaction changes with the cell’s growth conditions and in response to specific kinds of stress. The *E. coli* chromosome, with a linear size of 1.5 mm, occupies a volume of $0.1\text{--}0.2\ \mu\text{m}^3$ (the bare DNA volume is about a factor 20–30 smaller), which brings the need to study the folding geometry of the nucleoid (Stavans and Oppenheim 2006);
- (iii) Supercoiling and nucleoid organization play an important role in gene expression (Dillon and Dorman 2010). For example, during rapid growth, while several chromosome equivalents are present in the cell due to multiple ongoing replication cycles, an increased production of some NAPs is observed, compacting the chromosome and probably giving rise to specific transcription patterns in a way that is not yet fully characterized.
- (iv) RNA polymerase, the nucleoid-bound enzyme responsible for gene transcription, is concentrated into transcription foci or ‘factories,’ which can affect the nucleoid structure by bringing together distant loci (Grainger *et al* 2005, Jin and Cabrera 2006).
- (v) For cells that are not replicating the genome, the position of genetic loci along the chromosome is linearly correlated with their position in the cell (Breier and Cozzarelli 2004, Viollier *et al* 2004, Wiggins *et al* 2010). The exact subcellular positioning of different loci varies in different bacteria (Toro and Shapiro 2010).
- (vi) During replication, daughter chromosomes demix and segregate before cell division. In *E. coli*, the two arms of the chromosome are segregated in an organized <left–right–left–right> asymmetric fashion from the center of the cell (Nielsen *et al* 2007, Toro and Shapiro 2010, Wang *et al* 2006). Replicated chromosomal loci are thought to be immediately recondensed, as they appear to preserve the linear arrangement while they are moved in opposite directions to assume their final position in the incipient daughter cell (Thanbichler and Shapiro 2006, Thanbichler *et al* 2005).

In short, the nucleoid’s physical organization plays a major role in the most important cellular processes, such as cell division, DNA replication, and gene expression. Explaining these links is a long-standing open problem in microbiology. Today, it can be revisited with new quantitative experiments, including both the ‘-omics’ approaches and more sophisticated and controlled experimental techniques allowing the analysis of nucleoids both *in vitro* and *in vivo*. This is paralleled by a renewed interest in the quantitative characterization of bacterial physiology (Scott *et al* 2010, Scott and Hwa 2011, Zaslaver *et al* 2009), of which the nucleoid constitutes a fundamental part. Also note that the relative chromosomal positioning and the orientation of genes are subject to natural selection on evolutionary time-scales

as shown by the comparison of the genetic maps of different bacteria (Ochman and Groisman 1994, Rocha 2008).

Consequently, the field is blossoming with a new wave of studies, hypotheses and findings. While many new pieces of evidence are available, the challenge of building coherent pictures for physical nucleoid organization and its role in the different cell processes remains open. Our scope here is to present the main hypotheses and the experimental and modeling tools that have been put forward in order to understand the physical aspects of nucleoid organization, and give the interested reader an ordered account of the known facts.

3. Measurements

We start by reviewing some of the salient experimental findings at the scale of whole-nucleoids, with a particular emphasis on the more recent results. The evidence that we will discuss emerges from a combination of experimental biology, biophysics, high throughput biology and bioinformatics approaches (see box 2). Most of the studies reviewed here are based on *E. coli* or *Caulobacter*.

3.1. Chromosome spatial arrangement and compartmentalization

Strikingly, the intracellular localization of a given chromosomal locus in a cell is remarkably deterministic, as revealed by fluorescent tagging of chromosomal loci on *E. coli* and *Caulobacter crescentus* (Liu *et al* 2010, Nielsen *et al* 2006, Niki *et al* 2000, Viollier *et al* 2004, Wang *et al* 2006, Wiggins *et al* 2010). The series of chromosome segments is localized along the long axis of the cell in the same order as their positions along the chromosome map, with the interlocus distance typically linearly proportional to (arclength) genomic distance. The precise location of individual loci varies in the known bacteria, and might depend on DNA-membrane tethering interactions (Toro and Shapiro 2010). A recent microscopy study on *E. coli* (Meile *et al* 2011) considered the positioning of the chromosome in the short-axis section of the cell. They found that the Ter region occupies the periphery of the nucleoid, at a larger distance from the longitudinal axis with respect to the rest of the chromosome. In newborn or non-replicating cells, the two chromosome arms are spatially arranged such that loci on the left arm of the chromosome lie in one half of the cell and loci on the right arm lie in the opposite half, with the replication origin between them. It is tempting to interpret the resulting sausage-shaped structure as a chromosomal ‘fiber.’ In a recent study (Wiggins *et al* 2010), the cell-to-cell variability of loci positioning in non-replicating cells was used to estimate an internal elasticity, which, perhaps not surprisingly, appears to be much higher than expected from a naive estimate for a linear polymer.

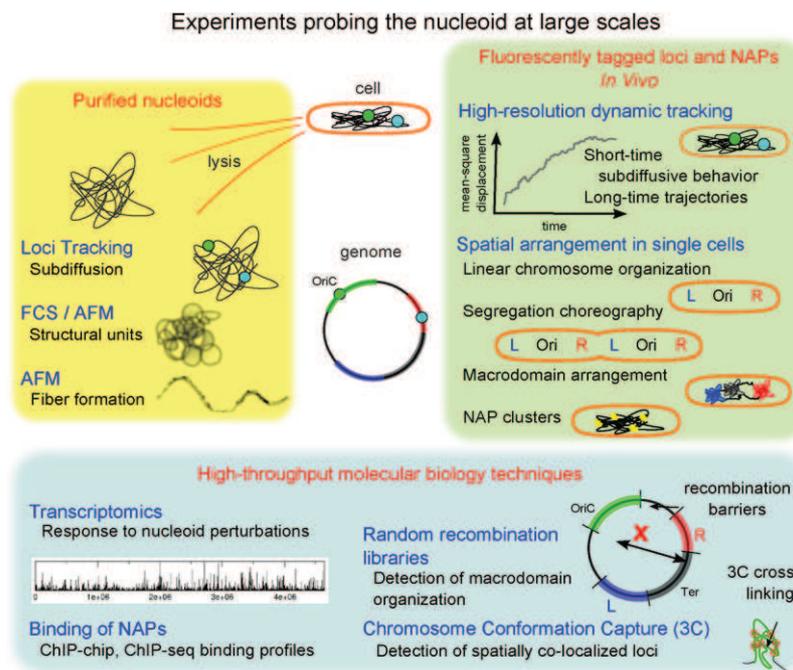
Even more recently (Umberger *et al* 2011), high-throughput chromosome conformation capture (3C) techniques have been used in combination with live-cell fluorescent tagging of loci, in order to determine the global folding architecture of the *Caulobacter crescentus* swarmer cell genome.

These data indicate that a chromosomal fiber exists also in this case, spanning the whole chromosomal ring. Additionally to the linear spatial arrangement of loci according to their chromosomal coordinate, loci of the left chromosomal arm tend to be very proximal to symmetric loci on the right arm. The resulting structure is a compressed ring-like fiber, which, the authors argue, typically takes an eight shape, free to roll around the long cell axis. They also find that the symmetry in the cross-chromosomal arm interactions is determined by the protein-dense attachment point to the cell membrane at the old pole of the cell, triggered by the binding of the ParB protein to its target parS binding sites. Moving the parS pole-anchoring site by 400 Kb along the chromosome (but not the replication origin) determines a sliding of the whole interaction structure, as in a tank crawler. This sliding is slightly asymmetric, suggesting the presence of supplementary attachment points between chromosomal arms or between the chromosome and the cell body.

Another important recent discovery, consistent with the mentioned correlation between chromosome arms and cell halves, is the existence of ‘macrodomains’ (Espéli and Boccard 2006, Moulin *et al* 2005, Valens *et al* 2004) often described as chromosomal isolated compartments. The first evidence in this direction (Valens *et al* 2004) came from measurements of the recombination frequency between loci, see figure 1(a). All else being equal, this should be proportional to the probability that the two chromosomal segments come into contact within the cell. For a well mixed polymer, the recombination frequency should be uniform. However, experiments show a highly non-uniform pattern, compatible with a compartmentalized structure with clear boundaries. Four macrodomains of a few hundred Kb in size have been identified, corresponding to regions surrounding the replication origin and terminus, and to two symmetric regions at the edges of the Ter macrodomain, see box and figures 1(c)–(e). The remaining ‘non-structured regions’ appear to have different physical properties. Subsequent studies have confirmed the presence of macrodomains and measured their dynamics using fluorescently labeled loci (Espéli and Boccard 2006, Espéli *et al* 2008, Lesterlin *et al* 2005), see figures 1(b) and (d). While the molecular mechanisms responsible for this level of organization are not yet clear, the same authors also found that the Ter macrodomain appears to be condensed by a single DNA-binding protein (MatP, figure 2) with a small set of specific binding sites (Mercier *et al* 2008). Other proteins with macrodomain-specific DNA-binding properties have recently been identified (Cho *et al* 2011, Dame *et al* 2011, Sánchez-Romero 2010, Tonthat *et al* 2011), and appear to be conserved in related bacteria. In view of the results from this thread of work, macrodomains might be seen as a process of microphase separation triggered by specific protein binding. Interestingly, as mentioned above macrodomain-like regions also emerge from independent large-scale genomic data (Berger *et al* 2010, Mathelier and Carbone 2010, Scolari *et al* 2011).

During replication, the chromosomes segregate following a well-defined ‘choreography,’ which has been the subject of multiple studies (Berlatzky *et al* 2008, Jun and Wright 2010, Toro and Shapiro 2010). While segregation is not the main

Box 2. Several available experimental techniques can probe the nucleoid at large scales.



Advanced microscopy together with cell-biological techniques yields information about structure and dynamics of the nucleoid (right). *High-resolution tracking* of tagged loci allows measurement of the local viscoelastic properties of the nucleoid. *Static configurations* of chromosomal loci in fixed cells allow determination of their spatial arrangement within non-dividing cells and following cell division. Dynamic tracking at long time scales gives information on the chromosome's '*choreography*' followed over a cell cycle and on the macrodomain subcellular arrangement. Fusions of NAPs with fluorescent proteins also enable evaluation of their *localization* within the nucleoid.

Nucleoids can be purified and manipulated outside of a cell in order to access more directly their biophysical properties (left). This procedure implies release of confinement and crowding, and dilution of binding proteins. As a result, purified nucleoids are several times larger in radius than the size of a cell. Traditionally, purified nucleoids were imaged by electron microscopy, showing a ramified plectonemic structures. More recently, investigators have concentrated on characterizing their organization as polymers *tracking of labeled loci*, fluorescence correlation spectroscopy (*FCS*) and probing them mechanically (*AFM*).

In addition, information on nucleoid organization can be obtained from high-throughput experimental datasets (Bottom). *Transcriptomics* (using sequencing or microarrays) can be used to probe transcriptional response to nucleoid perturbations, such as NAP deletions, changes in the average level of supercoiling, or local release of a plectonemic loop. Another important source of data is *protein occupancy*, for example by NAPs, and its correlation with gene expression. This information is obtained both by microarray (ChIP-chip) and by next-generation sequencing techniques (ChIP-seq). *Recombination* has been used to define macrodomains, as compartments within which recombination between chromosomal segments was more likely than recombination with segments laying outside of the compartment. Finally, *chromosome conformation capture* (3C) techniques probe the spatial vicinity of pairs of chromosomal loci in the (average) cell. They can be combined with sequencing in order to produce high-throughput data sets (Hi-C).

focus here, it is useful to discuss it briefly, as the existing approaches to this problem (both experimental and theoretical) are intimately linked with chromosome organization and will be mentioned in the following. Specifically, spatial reorganization of the segregating chromosome arms appears to preserve qualitatively the relationship between loci distance along the chromosome and in the cell. Moreover, reorganization ensures that the two replication forks remain in opposite halves of the cell during replication and that the relative orientation of the two reorganized nucleoids in pre-division cells is not random.

Quite interestingly, the spatial separation of sister chromosomes is not a continuous process, but has been observed to proceed through 'snaps' (Espéli *et al* 2008, Joshi *et al* 2011), suggesting the existence of energetic or entropic barriers for separation, possibly overcome by active processes.

There is debate on what is the main driver for chromosome segregation: entropic repulsion forces due to strong confinement into a box of linear polymers have been proposed to explain this behavior at least in part (Jun and Wright 2010); in experiments on replicating *B. subtilis* the chromosome compaction and spatial organization have been hypothesized to result from non-equilibrium dynamics (Berlitzky *et al* 2008), rather than from an entropic repulsion process; in *Caulobacter crescentus*, a contribution from bidirectional extrusion of the newly synthesized DNA from the transcription complex has been postulated to contribute to chromosome segregation (Jensen *et al* 2001, Toro and Shapiro 2010). Experiments using inhibition of protein synthesis by chloramphenicol show that this produces nucleoids with a more rounded shape and induces fusion of separate nucleoid bodies (van Helvoort *et al* 1996). Upon release from protein synthesis inhibition, the

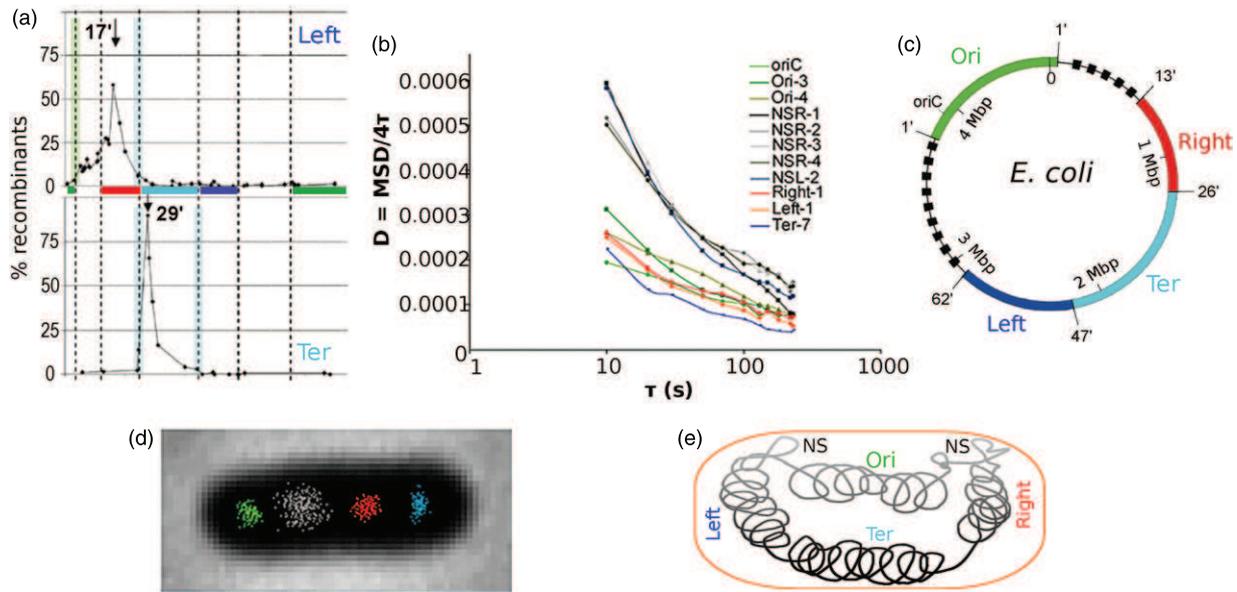


Figure 1. Chromosome compartmentalization and spatial arrangement of genes. (a) An example of the data that was used for the genetic definition of Right and Ter macrodomains, reprinted with permission from Macmillan Publishers Ltd. from Valens *et al* (2004), ©2004. The plot shows the frequency of recombination events between the genetic position indicated by the arrow and other positions probed along the genome (The x -axis indicates the base-pair coordinates of the chromosome). This should be uniform for a well-mixed polymer. In contrast, experiments show a highly non-uniform pattern, compatible with a compartmentalized structure with clear boundaries. Dashed vertical lines indicate the delimitation of macrodomains. Colored bars show the extent of the defined macrodomains. (b) Effective diffusion constants obtained from loci tracking experiments, reprinted with permission from John Wiley and Sons Ltd. (Espéi *et al* 2008), ©2008. The plot shows clear differences between the behavior of loci placed within macrodomains and in non-structured regions (NSR1-4, NSL-2). (c) After Valens *et al* (2004) (reprinted with permission from Macmillan Publishers Ltd. ©2004), graphical representation of the macrodomains and their boundaries within the *E. coli* genome. Structured macrodomains are indicated as colored arcs, black dashed arcs indicate non-structured regions. (d) From Espéi *et al* (2008) (reprinted with permission from John Wiley and Sons Ltd. ©2008), the genetic insulation of the macrodomains correlates with spatial insulation in subcellular territories. The positions of 10 foci were superimposed according to the barycenter of their trajectory during 30 intervals of 10 s at the home position. Foci from tags at OriC, NSR-3, Right-2 and Ter-6 were plotted. (e) Schematic of macrodomains' localization within the cell. Ter is localized at the periphery of the nucleoid, toward the cell membrane (Meile *et al* 2011) (reprinted with permission from Biomed Central, ©2011).

two nucleoids reoccupy the DNA-free cell independently of cell elongation (Helvoort *et al* 1998). The control of the segregation mechanism by protein synthesis processes could be indirect: ongoing protein synthesis can affect nucleoid compactness and segregation at multiple levels, including the decrease in the amount of enzymes modifying the topology of the DNA or carrying out transcription and DNA replication. The idea that membrane protein synthesis activates nucleoid segregation directly has also been proposed (Norris 1995). (In bacteria the presence of cotranscriptional translation creates a direct physical link between the genome and the membrane (Woldringh and Nanninga 2006).) Also note that current arguments based on segregation by entropy and confinement might turn out to be inconsistent with interpreting macrodomains as the result of a microphase separation, since a variety of interactions, and specifically protein–DNA-binding processes could play an important role in defining the free energy of the nucleoid.

3.2. Supercoil domains and nucleoid-associated proteins

At smaller scales, the circular chromosome of *E. coli* is organized in plectonemic loops, or ‘supercoil domains.’ Those regions are separated by topological barriers formed by NAPs such as Fis and H-NS, see figure 3. These proteins bridge

two strands of DNA by binding to both, and prevent the propagation of torsional energy. As a result, they also prevent the spreading of uncontrolled effects on gene expression in the case of accidental DNA breaks or mechanical strain, caused for example by advancing replication forks (Postow *et al* 2004, Skoko *et al* 2006, Stavans and Oppenheim 2006).

Multiple NAPs have been identified, each with its specific binding properties (reviewed in Luijsterburg *et al* (2006)). Moreover bridging double strands can change the local shape of DNA inducing bends or hinges and form nucleoprotein filaments. Additionally, NAPs often have multiple DNA-binding modes which might be dependent on physiological factors. A good example of this behavior is H-NS. Its binding results in DNA–H-NS–DNA bridges, but also forms a rigid nucleoprotein filament which could act as a zipper *in vitro* (Amit *et al* 2003, Dame *et al* 2006, Wiggins *et al* 2009). It seems that the nucleoprotein filament formation may be important, as it has been found to be a structure shared by other NAPs including HU (van Noort *et al* 2004) and StpA (Lim *et al* 2011). Biologically, this could hypothetically provide a mechanism for environmental sensing by NAPs. It is thus possible that our current understanding of NAP binding, being based on the limited number of conditions tested, is incomplete and generalizing a DNA-binding property to other solution conditions may be dangerous.

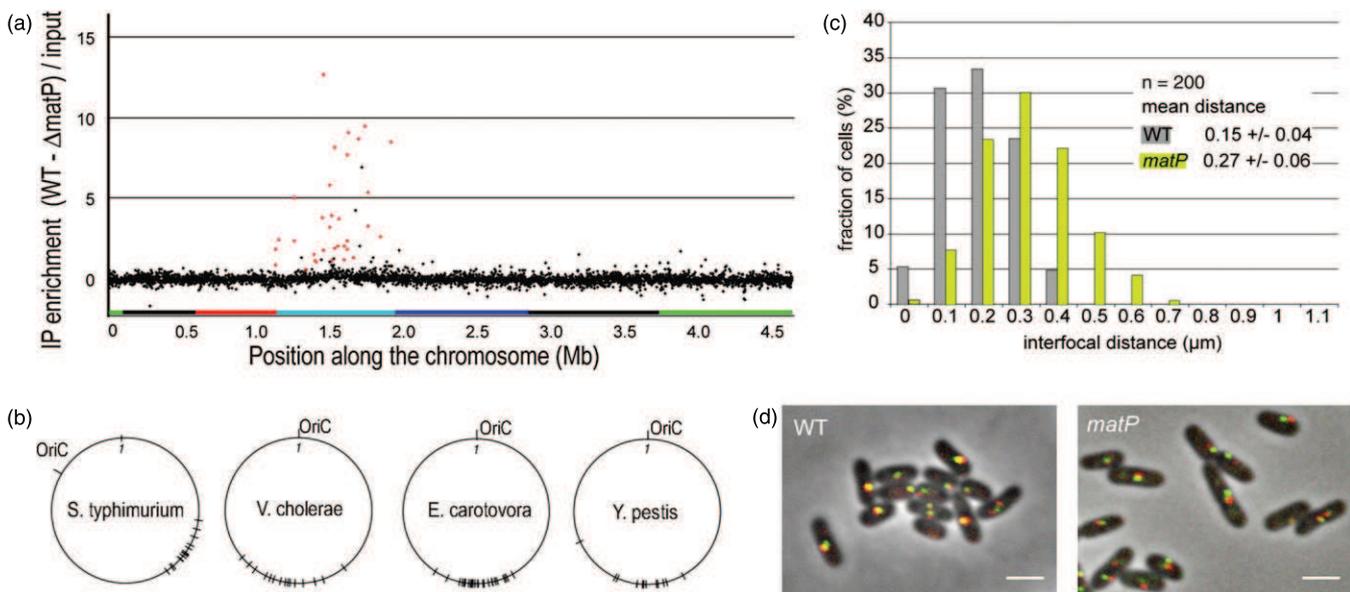


Figure 2. The protein *MatP* organizes the *Ter* macrodomain by specific binding. The circular chromosome of *E. coli* is divided into 4 macrodomains and 2 non-structured zones (see figure 1). (a) From Mercier *et al* (2008), ChIP-chip assays for *MatP* binding show a specific affinity of this protein with *Ter* macrodomains, indicating that the macrodomain is condensed by *MatP*, and suggesting that other macrodomains might be condensed by dedicated proteins with a small set of specific binding sites. (b) From Mercier *et al* (2008), the localization bias of *MatP* binding sites is conserved among enterobacteria, (*S. typhimurium* LT2 with genome size 4683 Kb, *E. carotovora* SCRI1043 - 5064 Kb), *Vibrio* (*V. cholerae* CI 2 - 961 kbp), and *Pasteurella* (*Y. pestis* KIM 4 - 600 kbp) species. (c) *MatP* controls DNA compaction in the *Ter* macrodomain. The histogram represents the proportion of cells with given interfacial distances between foci of two *Ter* MD markers in WT and *matP* deletion mutant cells. (d) Fluorescence microscopy images from the two experiments (scale bars 2 μm). These results indicate that the effect of *MatP* on foci colocalization is associated with compaction. (a)–(d) reprinted from Mercier *et al* (2008), ©2008, with permission from Elsevier.

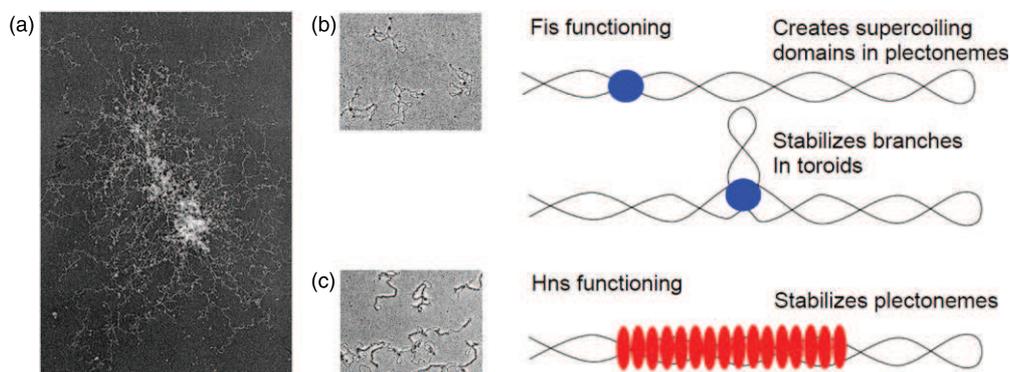


Figure 3. Branched plectonemic conformation of the genomic DNA molecule in 'supercoil domains' in *E. coli* and connection with NAPs. (a) From Postow *et al* 2004 (reprinted with permission from CSHL Press, ©2004), electron micrograph of a purified *E. coli* chromosome. The branched plectonemic structure is visible. From image analysis, Postow and co-workers measured a roughly exponential distribution for the length of supercoiled loops, with the average around 10–15 Kb. Note that the extraction and purification process might interfere with many of the properties of the conditions in which the nucleoid structure is naturally found. (b) Electron micrograph of Fis binding to plasmid DNA (from Schneider *et al* (2001)). This dimeric NAP stabilizes plectonemic branches of supercoiled DNA (schematized in the right panel). In particular, Schneider and co-workers measured a Fis to DNA ratio of 1 dimer per 325 bp DNA. (c) From Schneider *et al* (2001), electron micrograph of H-NS binding to plasmid DNA, elongated complexes (polymers) are formed (schematized in the right panel), presumably containing two DNA duplexes. Schneider and co-workers measured a ratio of H-NS to DNA of 1 molecule per 10 bp. (b)–(c) reprinted from Schneider *et al* (2001) with permission of Oxford University Press.

The structure of supercoil domains was studied in *E. coli* by Postow and co-workers, through analysis of the supercoiling-sensitive transcription of more than 300 genes following relaxation by restriction enzymes *in vivo*, and by electron microscopy (Postow *et al* 2004). They concluded that domain barriers may vary dynamically and/or across a population, but they follow an exponential length distribution.

The average domain size is ≈ 10 –15 Kb, implying the existence of about 200–400 domains (Postow *et al* 2004, Stavans and Oppenheim 2006). Branches of the same typical length are visible directly from electron micrographs of purified nucleoids (Kavenoff and Bowen 1976, Postow *et al* 2004). Thus, the genome topology may be visualized as a branched structure with supercoil domains that are subject to modulation

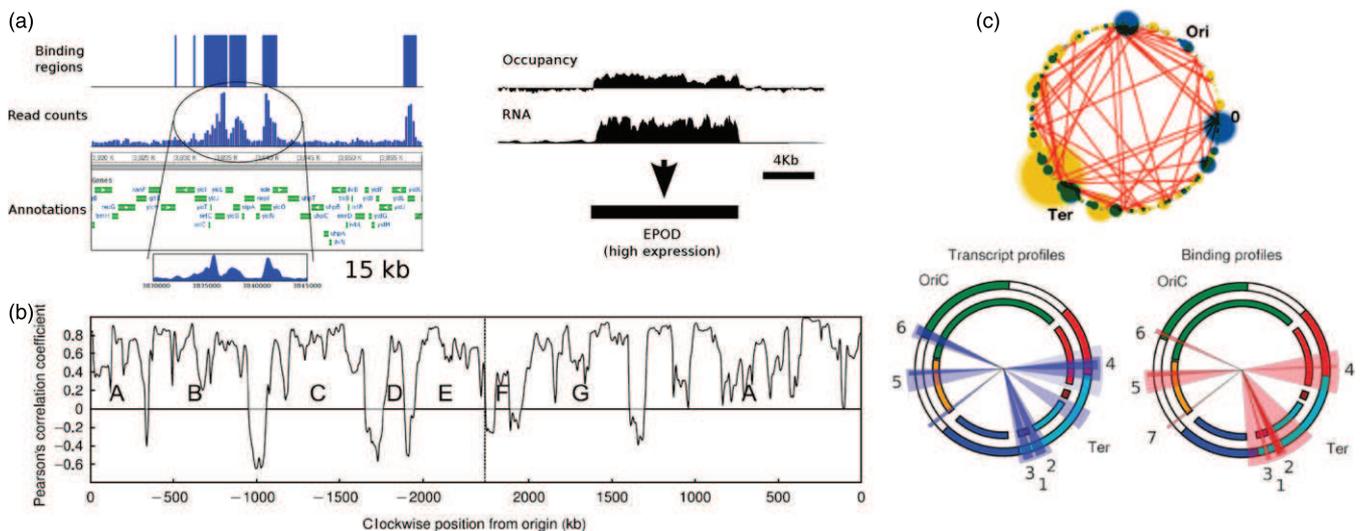


Figure 4. Examples of high-throughput data and bioinformatics analyses concerning nucleoid organization. (a) Left panel: ChIP-seq binding profile of H-NS, plotted using data from Kahramanoglou *et al* (2011); right panel: sketch representing an example of extended protein occupancy domains detected by Vora *et al* (2009) (and, in the example, associated with high expression). These polymer-like nucleoprotein complexes are presumably related to nucleoid organization. (b) Chromosomal sectors emerge from the local correlation (plotted here) between expression levels for wild type in log-phase growth and a codon bias index, related to translation pressure (from Mathelier and Carbone (2010), reprinted with permission from Macmillan Publishers Ltd: Molecular Systems Biology, ©2010). These sectors correlate well with macrodomain organization. (c) Top panel: effective transcriptional regulatory network (red links) and areas of influence of Fis and H-NS (colored circles) obtained from transcriptomics experiments combining NAP mutants and perturbations in supercoiling background (from Marr *et al* (2008), Reprinted with permission from BioMed Central, ©2008). Bottom panel: clusters of transcriptional response to nucleoid perturbations (left, data from Marr *et al* (2008) and similar experiments) and NAP binding (right) correlate with the organization of macrodomains (outer arcs) and chromosomal segments (inner arcs) of the genome (from Scolari *et al* (2011), reproduced with permission from The Royal Society of Chemistry).

by NAPs, and active processes such as DNA transcription and DNA replication.

The factors responsible for establishing the boundaries of supercoil domains and the determinants of domain size and number are still largely unknown. While H-NS and Fis, along with MukB, the analog of the eukaryotic SMC (structural maintenance of chromosomes) protein, could be involved in stabilizing pletonemic conformations, their precise roles and importance in this context have not yet been established in a definitive fashion, either *in vitro* or *in vivo* (Dillon and Dorman 2010, Grainger *et al* 2006, Maurer *et al* 2009, Skoko *et al* 2006).

In contrast, a consistent amount of information on the binding of NAPs under different conditions and its effects on the cell state is available from high-throughput experimental techniques (figure 4.). NAPs can modulate the nucleoid conformation structure in response to changes under environmental conditions (Luijsterburg *et al* 2006). This can result in large-scale changes in gene expression (Dillon and Dorman 2010). The local mechanical action of NAPs on DNA is often well-characterized by single molecule experiments (Luijsterburg *et al* 2006), which also lead to the observation of chromatin-like nucleoprotein ‘fibers’ (Kim *et al* 2004). Large-scale NAP binding data under specific growth conditions was obtained from high-throughput experiments involving microarrays (ChIP-chip) or sequencing (ChIP-seq) (Grainger *et al* 2006, 2007, 2008, Kahramanoglou *et al* 2011, Oshima *et al* 2006, Wade *et al* 2007). Furthermore, transcriptomics studies profiled the changes in gene expression

upon different nucleoid perturbations, such as NAP deletion and/or altered supercoiling (Berger *et al* 2010, Blot *et al* 2006, Bradley *et al* 2007, Marr *et al* 2008). Many of these data sets show linear regions of dense binding that often correspond to macrodomain boundaries, and associate with global or NAP-dependent transcriptional response and its correlation with codon bias (Mathelier and Carbone 2010, Scolari *et al* 2011).

The physical origin of this preference in binding and the gene expression changes at the boundaries of macrodomains is not precisely clear. One possibility is that macrodomain boundaries might be co-localized by NAP structures. In a recent interesting experimental study, Vora *et al* (2009) looked at protein occupancy along the genome regardless of protein identity. This work uncovered extended polymer-like domains rich in bound proteins (including NAPs) with an average length of 1.6 Kb, associated with transcriptionally silent or transcriptionally enhanced regions (and also with high intrinsic DNA curvature.)

When cells enter the stationary phase a radical, global condensation of the nucleoid occurs. It is believed that this is a mechanism via which the cell can protect its DNA under harsh conditions (Kim *et al* 2004). AFM studies have shown that the structure of the DNA differs at the supercoiling level (Kim *et al* 2004) and that action of Dps and CbpA, the NAPs that replace Fis in this growth phase, is quite different. The Dps and CbpA proteins produce compact aggregates (which can protect DNA from degradation by nucleases) rather than binding to distributed sites as Fis does (Cosgriff *et al* 2010). Interestingly, the action of Fis counters Dps-induced

compaction through a transcriptional response affecting the expression of topoisomerase and gyrase (Ohniwa *et al* 2006).

3.3. Compaction by molecular crowding, specific proteins, transcription factories and confinement

A different question concerns identifying the main factors contributing to nucleoid compaction and organization. The main candidates are macromolecular crowding, electrostatic self-attraction, supercoiling and nucleoid proteins.

Macromolecular crowding, or the high concentration of macromolecules present in the cytoplasm, is generally believed to be an important determinant on the basis of theoretical arguments (de Vries 2010, Odijk 1998), which predict a possible phase separation mechanism between nucleoid and cytoplasm. Note that the generic term ‘macromolecular crowding’ might include depletion interactions (see below), together with a number of additional effects of entropic and energetic nature.

DNA condensation by crowders can be observed *in vitro* under very controlled and well-understood conditions, for example by experiments using dextran or PEG, demonstrating that naked DNA can be directly condensed by these crowders (Estevez-Torres and Baigl 2011, Huang *et al* 2007, Xu and Muller 2012, Zhang *et al* 2009). Similar (but less controlled) behavior is shown by purified nucleoids (Zimmerman 2004). At the same time, experimental studies on isolated nucleoids obtained from mutants lacking various NAPs (Zimmerman 2006a) suggest that the effects of crowding on compaction are substantial and independent of the NAP composite background. It has also been suggested that the action of NAPs could be aimed at antagonizing compaction rather than compacting the nucleoid (Zimmerman 2006b). However, this must be a complicated, combined effect involving forces of different nature. In the absence of crowding and confinement, it is obvious that the radius of gyration of the genome would be smaller if it were organized, e.g. in a branched structure of plectonemic loops stabilized by DNA-bridging NAPs (Postow *et al* 2004, Trun and Marko 1998).

As a particular case of crowding effect, it has been proposed that the (attractive) depletion interactions, well known in colloid science, might play an important role in chromosome organization (Marenduzzo *et al* 2006a). This force is due to reduction in total solvent excluded volume upon formation of a molecular complex. Depletion interactions are consequential when large molecular assemblies are formed in the presence of smaller particles. Under fast-growth conditions, genome-bound RNA polymerase is localized into a few transcriptionally active foci or ‘transcription factories,’ (Cabrera *et al* 2009, Jin and Cabrera 2006), analogous to the eukaryotic case (Marenduzzo *et al* 2006a, Marenduzzo *et al* 2006b). Depletion interactions were suggested to explain the formation of these macromolecular assemblies (Marenduzzo *et al* 2006b). Interestingly, the formation of these foci has been associated with the presence of the NAP protein HU (Berger *et al* 2010). We add that a similar argument might hold for the local compaction of the surroundings of OriC, rich in ribosomal RNA producing

regions, in a macrodomain-microphase. In other words, ribosome-rich ribosomal RNA transcripts, attached to the genome through RNA polymerase, could help compact the Ori region. If this should be the case, the compaction properties of this region would change with the number of ribosomes being synthesized, i.e. with growth rate and translation efficiency (Scott and Hwa 2011). (One can also speculate that, for the same reason, newly replicated DNA could be sequentially aggregated during replication.) On one hand, experiments show that transcription of ribosomal RNA operons (which are generally located in the Ori macrodomain) is related to the compaction of nucleoids observed upon inhibition of translation (Cabrera *et al* 2009). On the other hand, these processes must be intersected with the (non-entropic) binding of NAPs, given the evidence connecting specific NAPs to the compaction of the Ter macrodomain (MatP) and indirectly to the effect of the HU protein on organization of the Ori macrodomain.

In a very recent study Wang *et al* (2011a) systematically addressed the chromosomal localization and role in spatial organization of the nucleoid of five major NAPs (HU, Fis, IHF, StpA, H-NS) using fluorescent protein fusions and super-resolution fluorescence microscopy. Under the growth conditions tested all the proteins showed scattered distributions in the nucleoid, except for H-NS, which seemed to form (on average) two distinct foci per chromosome copy, bringing together different (even distant) H-NS targets, see figure 5. Thus, H-NS should be added to the list of NAPs with a compacting action on the nucleoid associated with the formation of specific foci. The long-range interactions between H-NS binding targets were validated by 3C, and show no apparent coherence with the macrodomain structure; loci pairs that are near to H-NS targets but are not targets show no 3C signal and wider distributions of subcellular distances (evaluated with microscopy). In general, DNA associated with many NAPs has a much larger surface, which should enhance the depletion interactions. For instance, DNA coated with an H-NS nucleoprotein filament will have a diameter of about 20 nm instead of the 2 nm of naked DNA. H-NS nucleoprotein filament formation could strongly enhance the depletion attractions with respect to other NAPs not forming filaments, and filaments formed at remote locations on the contour of the chromosomal DNA could find each other in a crowded environment. This may explain the results from super-resolution imaging mentioned above (Wang *et al* 2011b). We note again that this is one of the counter-intuitive aspects of molecular crowding, since one might think that it would be easier to encounter another filament if there were no ‘obstacles’. The confusion can come from thinking about diffusion, which is hindered in a crowded environment due to the higher effective viscosity, versus thermodynamics, where the depletion interactions increase the probability of observing two nucleofilaments in contact at equilibrium. Adopting a simple Kramers-type model for the kinetics, it is not obvious whether the rate of association would go down due to the prefactor or go up due to the lower free energy minimum. Implicit in the entire discussion implicating crowding in aggregation in this cellular context is

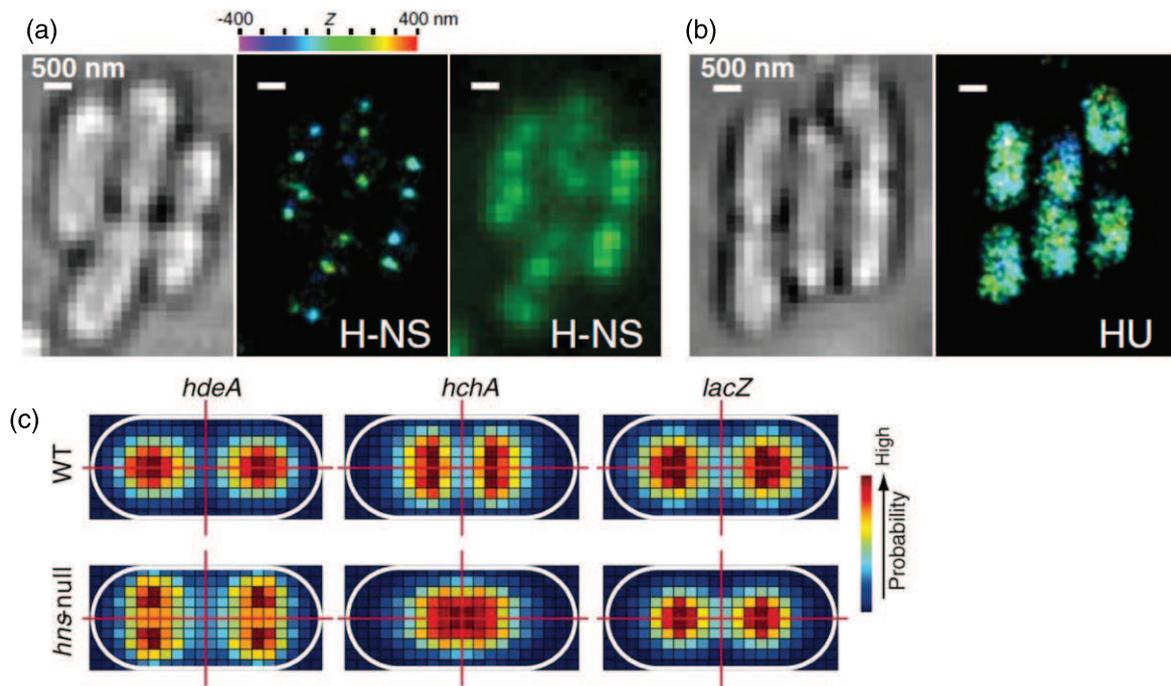


Figure 5. Effect of H-NS on chromosome compaction. From Wang *et al* 2011a, Reprinted with permission from AAAS. H-NS was shown by Wang and coworkers to form a small set of foci in the cell, which bring together distant H-NS targets. Together with MatP (figure 2), this evidence proves a role of drives of energetic origin in chromosome compaction. (a) Super-resolution fluorescence imaging of fluorescent protein fusion of H-NS showing compact H-NS clusters in the nucleoid. The *E. coli* cells are shown in the bright-field image (left). The z coordinate of each localization is color-coded (top bar). In comparison, a conventional fluorescence image of the same cells is shown (right). (b) Scattered distribution of HU in the nucleoid. Left: bright-field image; right: super-resolution image. Similar diffuse distributions were observed for Fis, IHF and StpA. (c) Effect of deleting H-NS on the subcellular distribution of H-NS target genes (*hdeA*, *hchA*) versus non-targets (*lacZ*). The 2D histograms of the relative *hdeA*, *hchA*, and *lacZ* locus positions normalized to the cell dimensions are shown, the genes were fluorescently labeled. The first quartile of the cell plots the (color-coded) probabilities of finding the fluorescent locus in the particular position, the rest of the cell is filled with mirror images to aid the eye (grid size 100 to 200 nm). In each case, 2000 to 5000 gene locus positions were analyzed. H-NS deletion has little effect on *lacZ* distribution, despite this locus is only a few Kb apart from the closest H-NS binding site.

the idea that the distribution of nucleoprotein filaments is at near-equilibrium even though the cell is dividing rapidly.

Finally, depending on the degree of autonomous compaction, the confinement exerted by the cell wall might play a relevant role in nucleoid organization and segregation (Jun and Wright 2010). This is expected to be particularly significant under fast-growth conditions, where the genome needs to be highly accessible for transcription (and thus will not be condensed) and the amount of genome per cell is higher due to overlapping replication rounds (Nielsen *et al* 2007).

To summarize, the degree of condensation and the geometry of the nucleoid are strongly dependent on the growth phase and growth rate of the bacteria. Such changes may be modeled by means of equilibrium states, slowly evolving in accordance to ‘external’ control parameters (e.g. the concentration of the various NAPs). However, it seems likely that non-equilibrium processes are also important, and relevant aspects of the problem might be lost in attempting to describe the nucleoid condensation process purely in terms of an approach to thermodynamic equilibrium. For example, on the other side of the spectrum in terms of biological complexity, the recently found scale-invariant structure of the human genome (Lieberman-Aiden *et al* 2009, Mirny 2011) was suggested not to be the result of an equilibrium state, but

similar in nature to the so-called ‘crumpled globule,’ or ‘fractal globule,’ (Grosberg *et al* 1993) since loci that are near along the genome arclength coordinate are also physically proximal in three-dimensional space. This proximity contrasts with what happens in an (equilibrium) collapsed polymer, where the linear structure is completely mixed in the globule.

Let us quote a few experimental findings, some of which are very recent, supporting the role of non-equilibrium processes, specifically for bacteria. The initial conditions, i.e. the choreography of DNA replication, appear to play a central role in defining the final structure of the nucleoid (Daube *et al* 2010). In artificial *E. coli* strains with two distant origins instead of just one (Wang *et al* 2011b), the two origins initiate replication synchronously at the expected separate positions of the genetic loci associated with them. Replication forks move independently, indicating that replication does not occur in a single replication factory and that the replication machinery is recruited to origins rather than vice versa. Most importantly, in these experiments progression of replication plays a major role in determining the space-time pattern of locus segregation. The large-scale structure of the *B. subtilis* nucleoid (Berlitzky *et al* 2008) has been observed at various stages of the replication process. The newborn portions of the chain are compacted and sequentially conveyed toward the poles, resulting in an ordered, spiraling structure. A

strong correlation between space coordinate and genomic coordinate is preserved, similar to the linear behavior observed in *E. coli* (Wiggins *et al* 2010). A choreography of this sort is found also in *Caulobacter* (Jensen *et al* 2001). Finally, the large-scale spiral structure of the nucleoid of *Bdellovibrio bacteriovorus* (Butan *et al* 2011) also suggests a metastable steady state, sustained by cooperative motion and/or energy exchanges. It seems difficult to disregard the deterministic replication–segregation dynamics in describing such phenomenology (Breier and Cozzarelli 2004, Toro and Shapiro 2010).

3.4. Viscoelasticity and structural units

Tracking studies of fluorescently labeled chromosomal loci have evaluated *in vivo* dynamic properties of the nucleoid with fairly high time resolution, measuring for example the mean-square displacement (MSD) of the loci or the time autocorrelation function (Espéli *et al* 2008, Meile *et al* 2011, Weber *et al* 2010a). In general, these measurements give information on the local relaxation time scales of the nucleoid and its viscoelastic behavior, see figure 6. On the one hand, for large time-scales (Espéli *et al* 2008), loci mobility correlates well with macrodomain structure. In particular, the MSD saturates at the spatial scale of the macrodomain size. On the other hand, especially for smaller time scales, the MSD of a locus is seen to follow a power law: $\text{MSD} \equiv \langle (\vec{x}(s, t + \Delta t) - \vec{x}(s, t))^2 \rangle \approx c \cdot (\Delta t)^\alpha$ (where s is its arclength genomic coordinate) and the exponent α seems to be universally close to 0.4, independent of s (Weber *et al* 2010a). Perhaps surprisingly, an extra-chromosomal RK-2 plasmid showed the same behavior, while smaller RNA particles had a higher subdiffusive exponent. It must be mentioned that the localization of RK-2 plasmids appears to be highly regulated (Derman *et al* 2008, Kolatka *et al* 2008). The underlying viscoelasticity of the bacterial cytoplasm surrounding the nucleoid is still poorly understood. Some characterization has been approached *in vivo* via FRAP measurements of diffusing GFP (Konopka *et al* 2006).

The observed anomalous diffusion has been modeled using phenomenological approaches. There are a variety of dynamical models exhibiting anomalous diffusion, such as Langevin equations with time dependent viscosity (also equivalent to fractional Langevin equations), continuous time random walks and random walks over a fractal object (Burov and Barkai 2008, Condamin *et al* 2008, He *et al* 2008). Weber *et al* (2010a) and Weber (2010b) compared their data with the results obtained on the basis of the first two approaches, and the fractional Langevin equation gave a more satisfactory agreement.

The anomalous diffusion exponent *per se* does not give quantitative information on the geometry of the nucleoid. Physical measurements, possible on purified nucleoids (Cunha *et al* 2001, 2005, Romantsov *et al* 2007), can enrich the scenario. In particular, Romantsov *et al* (2007) have obtained, using fluorescence correlation spectroscopy, the time-dependent coarse-grained density distribution of purified nucleoids. They conclude that the polymer appears to

be composed of a set of ‘structural units’ defined by a measurable correlation length. The measurements were performed at varying degrees of supercoiling, induced by different concentrations of the chloroquine drug. The size of the structural units was found to vary from 50–100 Kb in high (positive or negative) supercoiling to 3 Kb at zero supercoiling. The diameter of the purified nucleoid varied from 2.5 μm in high supercoiling to 3.5 μm in low supercoiling. The authors also estimated the typical diameter of the structural units from the diffusion constant (obtained from the decay of the fluorescence autocorrelation function) and Stokes–Einstein’s relation. Perhaps surprisingly, the resulting size of structural units was near 70–80 nm regardless of supercoiling. Thus the emerging picture for the unconfined genome is that of a string of ~ 100 highly dense ‘beads’, each containing ~ 100 Kuhn lengths (effective independently jointed elementary polymer segments) of DNA each. These values apply in the presence of supercoiling but in the absence of crowding and confinement effects, as for purified nucleoids most of the cytoplasmic (and probably a considerable part of the DNA-binding) proteins are probably diluted away.

4. Models

We will now review a few modeling approaches put forward in recent years, and point to some more classic work in polymer physics that we believe could be relevant in this context. As the reader will have observed, the wealth of existing experimental results is appealing on the one hand, but, on the other hand, does not offer any clear grasp on a small set of relevant ingredients necessary for building coherent physical descriptions of the nucleoid. Consequently, the approaches adopted in the literature are highly diverse and heterogeneous in terms of premises, methods, ingredients and points of view.

The notable efforts to understand entropic aspects of chromosome segregation (reviewed in Jun and Wright (2010)) have revived the studies on entropic forces of single and multiple confined polymers dating back to DeGennes (1979), Edwards and Freed (1969), Jung and Ha (2010), Jun and Mulder (2006). As we have pointed out above, the role of bound proteins on nucleoid entropy is typically disregarded in these arguments.

A comprehensive review of the literature on confined DNA in different contexts is provided in Marenduzzo *et al* (2010). Considering this approach, there remains the open issue that the observed segregation and compaction times might be too short to be compatible with an entropic process. For linear polymers this time has been evaluated by molecular dynamics simulations to scale like the square of the number of monomers, N^2 , which, for sufficiently large N , will be smaller than the $O(N^3)$ chain diffusion time (Arnold and Jun 2007). However, it is not straightforward to use these scaling relations for empirically relevant estimates. Moreover, these estimates will depend on the model used to represent the structure of the nucleoid and its correlation with the replication process, linking this problem to other unanswered questions. Overall, it seems likely that entropy is only part of the story, and

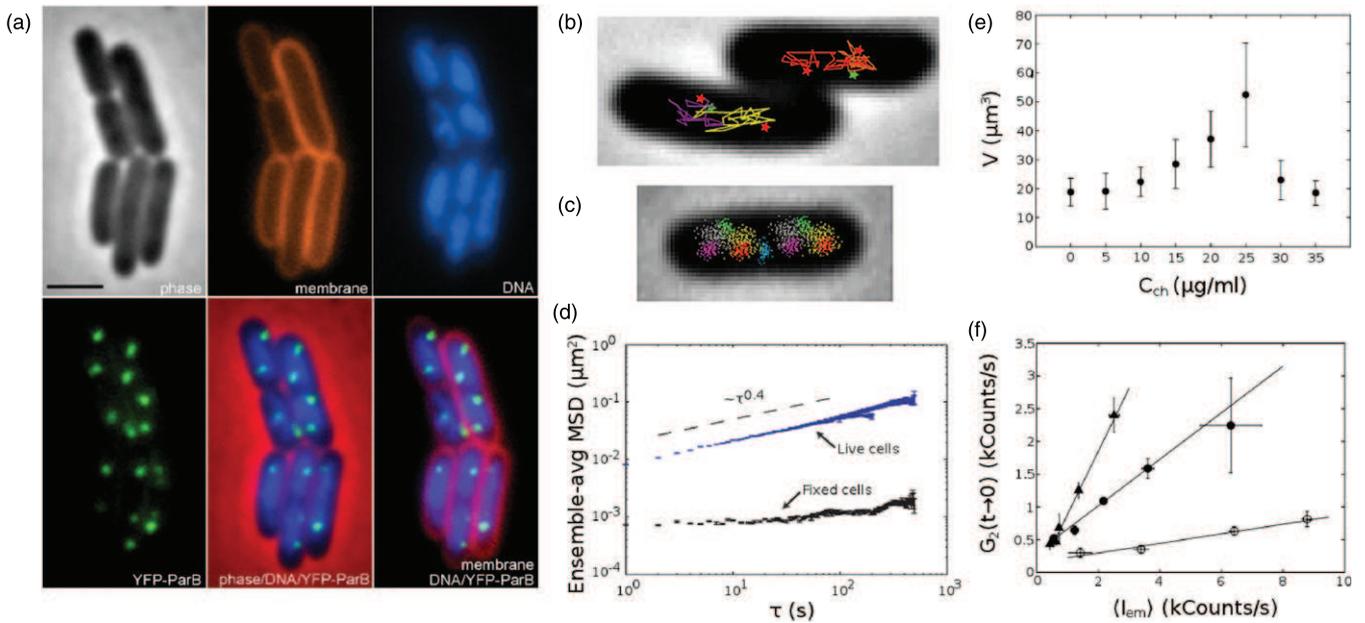


Figure 6. Optical techniques in nucleoid dynamic visualization. (a) From Meile *et al* (2011) (reprinted with permission from Biomed Central, ©2011), using phase contrast for cell visualization, membrane staining (FM 4-64), DNA staining (DAPI), and YFP-ParB for foci dynamic measurement, it is possible to produce ‘informed maps’ of the bacteria by overlaying different micrographs. (b) Movies can be used to follow foci dynamics. The plot, from Espéli *et al* (2008) (reprinted with permission from John Wiley and Sons Ltd. ©2008), shows a typical trajectory of a fluorescent focus, from which dynamical properties of the nucleoid can be inferred. (c) From Espéli *et al* (2008), reprinted with permission from John Wiley and Sons Ltd. ©2008. The trajectories of fluorescent dots define territories in which genetic loci are placed within the cell. (d) From Weber *et al* (2010a) reprinted, ©2010 by the American Physical Society. A measurement of the ensemble-averaged MSD for live and fixed cells using fluorescent loci. The authors showed that the dynamical exponent of the foci is universal (and close to 0.4). The observed anomalous diffusion has been explained phenomenologically as a consequence of the viscoelastic behavior of the nucleoid. We hypothesize that this could be a consequence of a fractal organization of the nucleoid similar to a ‘fractal globule’, see Discussion. (e), (f) Measurements on purified nucleoids, from Romantsov *et al* (2007), reprinted with permission from Elsevier, ©2007. Using fluorescence correlation spectroscopy, nucleoid volume and amplitude of FCS correlation functions have been measured at varying supercoiling (induced by different concentrations of chloroquine drug). The plots show that the nucleoid reaches a maximum volume and minimum FCS correlation at full supercoiling relaxation. The FCS correlation signal can be used to deduce the size of structural units of the polymer.

active/non-equilibrium processes of different kinds might play a role in chromosome segregation.

We will now turn our attention to work concerning nucleoid organization and cellular arrangement. Buenemann and Lenz have attempted to understand the linear arrangement of chromosomal loci in terms of a purely geometrical model (Buenemann and Lenz 2010), where a linear polymer, in the form of a string of blobs, is confined within a cylinder and locked at one or at a few loci. This constrained geometry obviously provides an ordering mechanism, as long as the blobs are large enough with respect to the cylinder’s diameter. This model makes predictions on the spatial arrangement of the chromosome in mutants of *C. crescentus* (Viollier *et al* 2004) and on the cell-cycle dependent ordering in *E. coli*. A very recent simulation study (Fritsche *et al* 2011) explains the linear ordering observed in *E. coli* as a product of confinement and entropic repulsion of a string of linearly arranged chromosomal loops. In order to show this, they represent the chromosome as a confined circular self-avoiding chain under the constraint that consecutive loops, identical in size, are distributed along the arclength coordinate, while the Ter region does not contain such loops. Their simulations show both linear ordering along the cell axis and Ter region occupying the outer periphery of the nucleoid (Meile *et al* 2011), as properties of the equilibrium

states. Intriguingly, the same study suggests that this linear-loop ordering could be a consequence of the transcription network organization. To support this point, they simulate a polymer where transcription factor–target pairs are coupled by attractive harmonic interactions, and show that the linear ordering is recovered. It is well known that transcription factor–target distances have a statistical tendency to be short along the chromosome (Warren and ten Wolde 2004).

Vettorel *et al* (2009) performed an abstract study motivated by the possible nature of the compartmentalization and the structural units of a generic (eukaryotic or prokaryotic) chromosome forming a crumpled or fractal globule mentioned in section 3 (figure 7). Specifically, they explore metastable collapsed states of polymers, where the total size scales as $N^{1/3}$ as in an equilibrium compacted globule, but a much higher degree of compartmentalization is present. At odds with the intrinsic disorder of the equilibrium globule, in a fractal globule, for any pair of loci separated by a chain length s , the distance $R(s)$ has the scaling behavior $R(s) \approx s^{1/3}$ (Mirny 2011). In other words, both the equilibrium globule (i.e. the equilibrium collapsed structure emerging from polymer self-attraction or unfavorable entropy of mixing) and the fractal globule have mass fractal dimension $D_f = 3$, but a generic volume (and in particular a blob in the DeGennes sense)

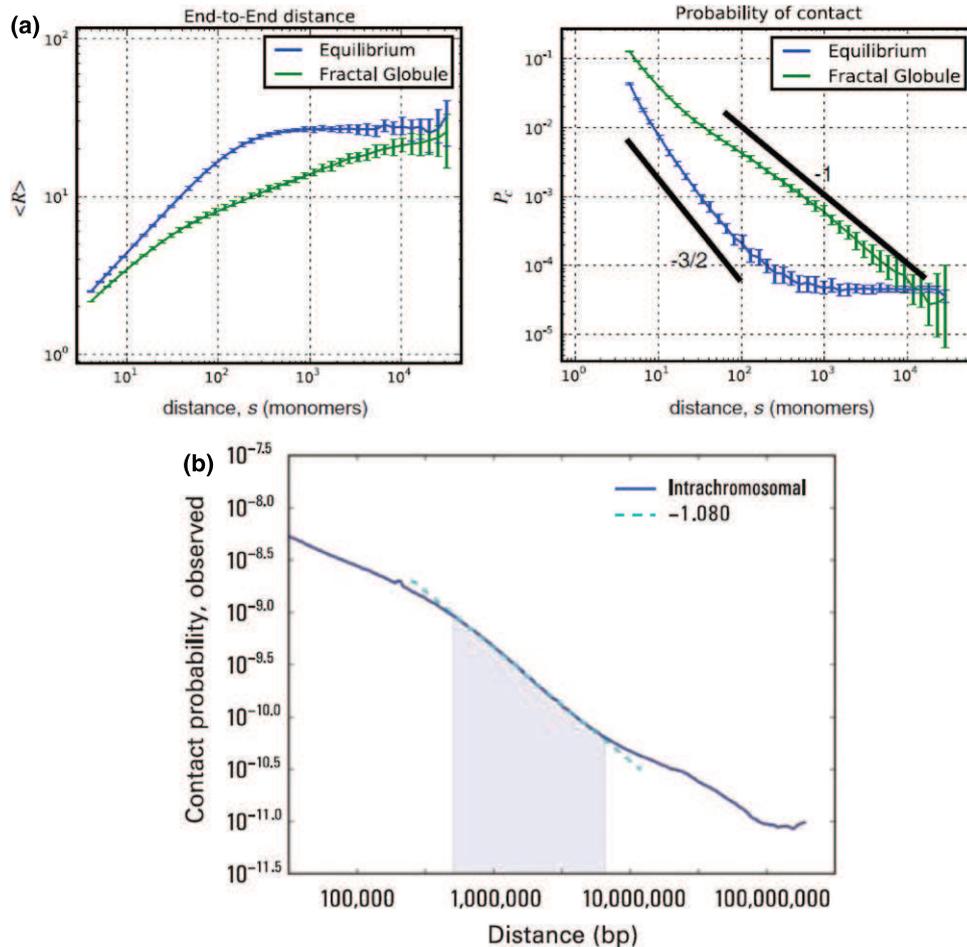


Figure 7. Main universal features of the ‘fractal globule’ model. (a) From Mirny (2011) (with kind permission from Springer Science + Business Media), root-mean squared end-to-end distance $R(s)$ and probability of a contact $P(s)$ as a function of the genomic distance s between the ends of a subchain of $N = 32\,000$ monomers, from simulations of a fractal globule and a collapsed (equilibrium) globule. Note the different scalings in the two situations. All random collapsed configurations of a polymer in two dimensions behave as a fractal globule, as topological entanglement is forbidden. This does not hold in three dimensions, as a large number of knots can be generated. In a kinetically constrained situation where knots are not present, this property is restored, and the resulting metastable configuration has the property that segments that are close in arclength distance are also close in space. It has been argued that this configuration might be relevant for eukaryotic chromatin within a range of length and time scales. (b) From Lieberman-Aiden *et al* (2009) (reprinted with permission from AAAS), contact probability as a function of genomic distance averaged across the genome (blue) of an eukaryotic cell obtained by the Hi-C technique. It shows a power law scaling between 500 kb and 7 Mb (shaded region) with a slope of 1.08 (fit shown in cyan). This analysis indicates that the eukaryotic DNA might be organized similarly to a fractal globule.

inside the equilibrium globule can include non-sequential segments, while in the fractal globule it contains a single sequential segment (Grosberg *et al* 1993). This structure can be understood as resulting from a process where condensation sequentially involves larger and larger scales in s , so that the genomic proximity is preserved in a scale-invariant fashion. As a consequence, distant portions of the chromosome will occupy different compartments within the globule.

In order to obtain a fractal globule in a simulation, a constraint preventing entanglement must exist (Vettorel *et al* 2009). To obtain this condition, Vettorel and co-workers considered a semi-dilute or concentrated solution of mutually disentangled (unconcatenated) rings. Also note that because of the constraint preventing entanglement during collapse, a fractal globule is generally larger in size than an equilibrium globule of equal chain length (while for both the total size scales as $R \approx N^{1/3}$). The fractal globule

configuration has proven to be relevant for the description of genomic DNA organization in non-dividing eukaryotic (human) cells (Lieberman-Aiden *et al* 2009, Mirny 2011). In particular, it has been found that the human chromosomes display this structure from $\Delta s \approx 500$ Kb to $\Delta s \approx 7$ Mb. This range of sizes might be relevant to bacteria that have genomes spanning a few Mb (also because the lower cutoff might be related to the fiber organization of chromatin, which in bacteria is different). Thus, as the authors speculate, the fractal globule description might be useful for the nucleoid as well. We will further discuss this point in the light of the available data in section 5.

We have already mentioned above the theoretical work on the role of macromolecular crowding on compaction de Vries (2010), Odijk (1998), and of depletion interactions on loop formation (and possibly on macrodomain organization (Marenduzzo *et al* 2006b)). A recent simulation study (Junier

et al 2010) has concentrated on the chromosome-shaping role of transcription factories. Considering a self-avoiding worm-like chain with a fixed hard-core repulsion radius, and short-ranged bridging protein complexes, they show that this system can take ‘micro-structured’ collapsed globule configurations, where bridging complexes cluster and regions of high and low densities of interacting sites coexist in a microphase separated thermodynamic state. More abstract analytical studies could provide a useful context for understanding the role of NAP binding (Diamant and Andelman 2000, Kantor and Kardar 1996).

Finally, some attention has been devoted recently to the role played by the branched pletonemic structure of the nucleoid (Odijk and Ubbink 1998, Ubbink and Odijk 1999), which is believed to have relevant implications for transcription (Dillon and Dorman 2010, Postow *et al* 2004). Provided the correct questions are formulated, this topic has the advantage of being placed within a strong framework developed in the past 30 years, building on the classical calculations of Zimm *et al* (1949) for considering arbitrarily ramified ghost chains as Gaussian networks, and obtaining their equilibrium properties (Farago and Kantor 2000, Graessley 1980, Sommer and Blumen 1995). For example, some recent work has focused on the induction of loops involving multiple polymer segments (Sumedha and Weigt 2008). Within this framework, reliable estimates for the self-avoiding case can be obtained with Flory-like arguments, taking into account the fact that branched polymers have higher internal topological complexity, which makes the repulsive interactions stronger than between linear or circular chains. The same approach is also possibly relevant for studying dynamic aspects of loci mobility (Dolgushev and Blumen 2009, Jasch *et al* 2003), which can be compared with simulations and more phenomenological models (Weber *et al* 2010b). Finally, a number of very refined results based on renormalization group/field-theoretical methods have been obtained in a more abstract context using the ‘randomly branched polymers’ model (or ‘lattice animals’), typically defined as the ensemble of all the clusters of connected sites (monomers) on a regular lattice. While this ensemble is probably very general, it is possible that these results have implications for questions related to the structure of the nucleoid. For example, recent work based on Langevin dynamics (Janssen and Stenull 2011) analyzes their collapsed regime, obtaining a fractal dimension $D_f = 2.52$, intermediate between the swollen chain ($D_f = 2$) and the fully compacted globular state ($D_f = 3$).

5. Discussion. Hypotheses and paradoxes concerning nucleoid geometry and dynamics

We would like to discuss here some speculations on the possible links between the experimental and theoretical results discussed above.

Let us start with an analogy to the geometrical organization of eukaryotic chromatin, where different geometrical features are observed at different scales, ranging from the known fiber organization up to the arrangement in preferred ‘chromosome

territories’ within the nucleus. In this case, experimental techniques such as chromosome conformation capture (3C) and its high-throughput variants (Lieberman-Aiden *et al* 2009), or FISH (fluorescence *in situ* hybridization, where fluorescent tags are attached to pairs of DNA loci) allow, for instance, to measure the mean absolute distance, R , and the contact probability, P_c , of two genomic loci at arclength distance s . For bacteria, these techniques entail a number of specific difficulties, but the data are starting to be available, as already mentioned (Umbarger *et al* 2011). However, the study by Umbarger and co-workers focuses on the large-scale nucleoid 3D architecture, rather than on more detailed properties of the interaction map. Apart from saturation at large genomic distances, $R(s)$ is typically found to increase with s (Mateos-Langerak *et al* 2009) with an approximate power-law behavior, $R(s) \sim s^\nu$. Here, ν is a scaling exponent, interpretable as $1/D_f$, which empirically varies with scale of observation and cell type in eukaryotes. If a stretch of length s of a polymer spans a region of size R , in D dimensions, it can occupy a volume $V \sim R^D \sim s^{\nu D}$. Thus, heuristically, one expects that the probability of one end of the stretch meeting the other scales as $P_c \sim 1/V \sim s^{-D\nu}$. Experimentally, for loci on the same chromosome, $P_c(s)$ decreases as a power-law $P_c(s) \sim s^{-1}$, for a set of length scales in the approximate interval 0.5–7 Mb for s (Lieberman-Aiden *et al* 2009), which provides evidence for a fractal globule-like organization, $\nu = 1/3$. This is confirmed by FISH data for $R(s)$ on a smaller range of genomic lengths (Mateos-Langerak *et al* 2009).

However, experimental data on $R(s)$ for chromatin are complex, as the ν exponent is cell-type specific and varies with genomic length, reflecting different degrees and modes of chromatin compaction. At short genomic distances ν is found in the range 0.2–0.6 at short distances, and $R(s)$ reaches a plateau (i.e. $\nu \sim 0$) at order 10 Mb genomic distances, because of chromosome territories (Barbieri *et al* 2011, Mateos-Langerak *et al* 2009, Shopland *et al* 2006). For the *E. coli* nucleoid, the only available quantitative data (Wiggins *et al* 2010) indicate that $R(s)$ might scale like s for non-replicating chromosomes at scales of 0.3–2 Mb.

In principle, a link between nucleoid geometry and the measured nucleoid local dynamics is expected. The anomalous diffusion of chromosomal loci in *E. coli* has been modeled in terms of fractional Langevin equations (Weber *et al* 2010b); such an approach correctly reproduces the temporal behavior of the loci, but disregards the geometry of the structure containing them. Very likely, for any polymer, this structure has a fractal character, and one would like to understand how this influences the motion of the loci. A minimal model would consider a relaxation equation over the fractal. For a purely self-avoiding polymer one has $D_f \approx 1.7$. In the case of a self-attracting polymer where attraction is screened by self-avoidance, i.e. at the θ point (DeGennes 1979, Grosberg and Khokhlov 1994), it is reasonable to assume a mass fractal dimension $D_f = 2$, as for a ghost chain. When the tendency toward compaction increases, one expects that D_f will increase accordingly.

In order to illustrate this point with an example, we can consider protein structures. Data from the Protein Data Bank (Berman *et al* 2000) for 200 proteins with a number

of amino acids ranging from $N \approx 100$ to $N \approx 10\,000$ give values of D_f from 2.3 up to 2.6 (Enright and Leitner 2005). On a larger scale, high-resolution x-ray spectroscopy has resolved the ribosome structure at the atomic level. Such data indicate (Lee 2006) that the heavier 50S unit is fully compacted, with $D_f = 3$, while the lighter 30S unit has $D_f = 2.8$; it has been argued that the sparser structure of 30S is compatible with a dynamic geometry, as required in the translation process. Folded proteins are generally described as an harmonic network, by the so-called Gaussian network model (Reuveni *et al* 2010), and we can try to apply a similar reasoning to the nucleoid. Close to the fully collapsed regime of a very long polymer such as a bacterial chromosome, where one expects $D_f \approx 3$, the harmonic approximation strictly does not apply, because hard-core repulsion acts against chain compression. The total energy can then be written as an harmonic ‘Rouse’ term, describing waves propagating along the chain, plus a self-attraction term and a self-avoidance term. The relaxation dynamics under such conditions (neglecting hydrodynamic interactions) has been studied within a continuum model approach by Pitard *et al* (1998). They find that the relaxation time τ of the globule scales with the polymer length N as $\tau \approx N^{5/3}$. Taking into account that the globule size R scales as $R \approx N^{1/3}$ (i.e. it fills space), this implies that $R^2 \approx (\tau^{3/5})^{2/3} \approx (\tau)^{2/5}$ (since $N \approx \tau^{3/5}$). This scaling appears to coincide with the experimental value for the anomalous diffusion exponent $\alpha = 0.4$ measured by Weber *et al* (2010a), previously quoted in the text. In other words, the Rouse subdiffusive dynamics of a collapsing (and thus off-equilibrium) globule follows the same scaling law as the observed local dynamics of nucleoid loci within a range of time scales. While this might be simply a coincidence, it leads us to speculate that the measured dynamic exponent for the MSD might be the consequence of a fractal-globule-like nature of the nucleoid, at least within a range of length scales.

This argument can be recast in more generic terms. Rouse polymer relaxation dynamics was originally explored by DeGennes (1976), who obtained the scaling relation $z = 2 + D_f$, where $z \equiv 2/\alpha$ is the so-called dynamical exponent. At the θ point (DeGennes 1979, Grosberg and Khokhlov 1994) where one has $D_f = 2$, the DeGennes’s relation gives $\alpha = 1/2$, the Rouse result for non-interacting chains. As the polymer dimension D_f increases, a smaller value of α is to be expected; in the compacted configuration, where $D_f = 3$, the relation gives $z = 5$, which is the result reported above. DeGennes’s work is based on scaling arguments, but is confirmed by field-theoretical methods (Wiese 1998) for two-body interactions.

To our knowledge, in the collapsed regime the relation has been proved only at the level of mean field (Pitard and Orland 1998) by modeling, in the spirit of a virial expansion, the effective interaction with an attractive two-body term and a repulsive three-body term.

In conclusion, if the nucleoid behaves like a fractal globule (i.e. an off-equilibrium polymer collapsing because of self attraction, but where entanglement is prevented by topological constraints), or more in general if it has fractal dimension $D_f = 3$, from mean field theory one expects the subdiffusion

exponent $\alpha = 0.4$. Conversely, if the DeGennes relation is valid, the experimental result $\alpha \approx 0.4$, observed in *E. coli* and in large plasmids (Weber *et al* 2010b), implies that the nucleoid fractal dimension could be $D_f \approx 3$.

To our knowledge the available experimental result that comes closer to a direct measurement of D_f deals with the MSD of fluorescently tagged replisomes (Reyes-Lamothe *et al* 2008). In the approximation of constant replication fork velocity along the mother DNA, replication time in this experiment and genomic arclength distance have equal scaling. Hence, neglecting the global movements due to chromosomal segregation, the replisome’s anomalous diffusion exponent α_R measures the effective fractal dimension $D_{f,R}$ of the replicating DNA. Specifically, the scaling $\text{MSD}(t) \sim t^{\alpha_R}$ implies $\alpha_R \sim 2/D_{f,R}$. Obviously, this measured $D_{f,R}$ in principle contains errors due to fluctuations of a stationary background as well as large-scale effects associated with coherent restructuring of the nucleoid. The latter processes are relevant for segregation, but in the initial phases of replication one can assume that they can be disregarded, as we are in the presence of a ‘weak perturbation’ of the stationary (non-replicating) structure. In such a case, the time fluctuations of the fork velocity and the steady-state anomalous diffusion of genetic loci would be the main corrections to be taken into account in order to estimate D_f from the measured $D_{f,R}$. Quite interestingly the experimental estimates of the exponent α_R from Reyes-Lamothe *et al* (2008) are $\alpha_R \sim 0.66$ and 0.58 for experiments with 30 s and 5 min time lapses, respectively. If these numbers were confirmed by further measurements and accurate data analysis, they would support the hypothesis that $D_f \sim 3$ for a range of chromosomal scales, independently on the reasoning presented above, based on the DeGennes scaling relation $z = 2 + D_f$.

Clearly, for the nucleoid one expects a structure with a range of fractal dimensions in different scale regimes, as suggested by the case of chromatin and the ribosome. The existence of a range of fractal dimensions is also supported by the microscopy results for fluorescent pairs of loci reporting a linear correlation between loci distance in the cell and along the chromosome (Wiggins *et al* 2010). The linear correlation could result from any sequentially ordered segregation process generating a uniform mass density. For example, one might consider a periodic winding of the chromosome (Képès and Valliant 2003, Mathelier and Carbone 2010), that could be produced by a segregation choreography of the type observed in *B. subtilis* as well as in *E. coli*. However, this needs to be reconciled with the observed subdiffusion. As for the case of chromatin, a hierarchical structure is very logical, since some chromosomal functions, such as transcription, replication and DNA repair, require a certain degree of plasticity, and are not compatible with full compaction at all scales and at all times. Thus, the linear correlation of loci subcellular position and genomic distance can be consistent with $D_f \approx 3$, but further work is needed to determine the range of spatial scales where these properties apply.

The main objection against the fractal globule as a long-lasting transient state supported by topological constraints is the ubiquitous presence of topoisomerases, DNA enzymes

able to cut and paste strands and thus easily resolve these constraints. An interesting theoretical study has focused on the entanglement of tethered rings (Marko 2009); it is argued that entanglements would ‘condense’, i.e. aggregate in space, in physically relevant situations, which, in the presence of enzymes, would facilitate further the resolution of topological constraints. This kind of objection holds for both the eukaryotic and the prokaryotic cases. It cannot be excluded that the non-equilibrium constraints leading to the fractal structure observed in Hi-C experiments are caused by something else, or more in general, and more plausibly, that a different physical process than simple topological constraints leads to the observed phenomenology. However, the generic reasoning presented above for connecting D_f and α might be robust with respect to these considerations.

Other approaches to chromatin organization aim at reproducing the interlocus distance $R(s)$ and the distribution of interacting loci $P_c(s)$ with alternative polymer models, such as a collapsing self-avoiding walk in a solution of organizing proteins which can bind and act as discrete self-attraction points, representing organizing proteins (Nicodemi and Prisco 2009). The spirit of this kind of study is to go beyond a dominant role of entropy, and take more seriously the ‘energetic part’ of the free energy, and in particular the organizing proteins. The consequences of this hypothesis are explored by analyzing the resulting equilibrium structures for the polymer. Nicodemi and co-workers have recently found that such a model polymer could be close to the θ point for empirically relevant protein concentrations, and small variation of the concentration of binding proteins around this state could recapitulate a considerable part of the observed phenomenology of nuclear eukaryotic DNA (Barbieri *et al* 2011).

Finally, as mentioned above, it is also worthwhile to consider whether equilibrium statistical mechanics is even the proper starting point to understand the structure of the bacterial nucleoid. Cells expend a considerable amount of energy maintaining steady-state, non-equilibrium environments. A classic example is the membrane potential, whose existence requires an elaborate mechanism for pumping protons through the membrane. Given that the genomic information is arguably the most important part of the cell, containing both the instructions for the current cell and the inheritable information for the next generation, it seems unlikely that bacteria have evolved such that the structure of the nucleoid is resigned to equilibrium.

6. Conclusions

To conclude, we briefly review some of the main features of the partial and emerging picture of the nucleoid, from the physics viewpoint. All these problems still need to be understood in a quantitative framework for bacterial physiology (Scott and Hwa 2011), and in particular for varying growth rates (and subcellular compositions) and during adaptation to different growth conditions (Muskhelishvili *et al* 2010).

A first problem that can be isolated is the explanation of its compaction/condensation properties. Likely mechanisms that

can influence (positively or negatively) nucleoid condensation (and more than one can be at play) include (i) supercoiling, bending and looping, in interplay with binding of NAPs, which can cause punctual or polymer-like links building aggregation foci (H-NS, MatP) and stabilize a ramified plectonemic loop structure (Fis), (ii) consequences of molecular crowding, in the form of both phase separation and depletion interactions, and (iii) (non-equilibrium) segregation after replication, which could be induced by different physical processes.

A second, related problem is the *geometry* of the nucleoid, which requires understanding how the subunits are arranged in the cell at different scales and times. It is likely that the organization principles in a given cell state (determined, e.g., by growth rate and growth phase) are different at different scales. At the micron scale, the experiments seem to converge toward a linearly arranged sausage-shaped structure, sometimes wrapped by the Ter region, and the main outstanding questions seem to relate to the physical mechanisms behind segregation and its choreography. Below this scale, the existence of macrodomains and transcription foci still elude a physical explanation, which could be microphase separation stabilized by short-ranged attractions of chemical (organizing proteins such as MatP) or of entropic (ribosome-induced depletion interactions) origin. At an even smaller scale, an organization in blobs or fibers seems to be equally elusive, despite the existence of numerous pieces of evidence for different aspects of NAP binding and plectonemic loop formation and stabilization.

Finally, it is important to point out that within the layered information given here there lies more than one unresolved question. For example, if macrodomains are microphases structured by protein binding, then certainly these proteins must play an important role in the configurational entropy of the nucleoid, which is not considered in the arguments concerning entropy-driven chromosome segregation. Also, if the genome is compacted (at least in a range of scales) in a fractal or conventional globule configuration by attractive interactions of entropic or energetic origin, this will considerably affect its entropy, and thus its mechanical properties, loci dynamics and the interactions between segregating chromosomes. Equally important, the supercoiling-independent size of structural units measured for purified nucleoids (whose size varies with supercoiling) appears challenging for theoretical explanations. While we are certainly far from a coherent and consistent physical description of the nucleoid, there is a clear abundance of existing data and many ongoing experiments merging quantitative biophysics and high-throughput molecular biology. These emerging results, together with the fragmented but partially successful modeling approaches, make us believe that we might be on the verge of resolving at least some of the existing issues regarding the physics of the bacterial nucleoid.

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References

- Alberts B, Bray D, Lewis J, Raff M, Roberts K and Watson J D 2008 *Molecular Biology of the Cell* 5th edn (New York: Garland)
- Amit R, Oppenheim A B and Stavans J 2003 Increased bending rigidity of single DNA molecules by H-NS, a temperature and osmolarity sensor *Biophys. J.* **84** 2467–73
- Arnold A and Jun S 2007 Time scale of entropic segregation of flexible polymers in confinement: implications for chromosome segregation in filamentous bacteria *Phys. Rev. E* **76** 031901
- Barbieri M, Chotalia M, Fraser J, Lavitas L-M, Dostie J, Pombo A and Nicodemi M 2011 Complexity of chromatin folding: the strings and binders switch model (submitted)
- Berger M, Farcas A, Geertz M, Zhelyazkova P, Brix K, Travers A and Muskhelishvili G 2010 Coordination of genomic structure and transcription by the main bacterial nucleoid-associated protein HU *EMBO Rep.* **11** 59–64
- Berlatzky I A, Rouvinski A and Ben-Yehuda S 2008 Spatial organization of a replicating bacterial chromosome *Proc. Natl Acad. Sci. USA* **105** 14136–40
- Berman H M, Westbrook J, Feng Z, Gilliland G, Bhat T N, Weissig H, Shindyalov I N and Bourne P E 2000 The protein data bank *Nucl. Acids Res.* **28** 235–42
- Blot N, Mavathur R, Geertz M, Travers A and Muskhelishvili G 2006 Homeostatic regulation of supercoiling sensitivity coordinates transcription of the bacterial genome *Embo. Rep.* **7** 710–15
- Bradley M D, Beach M B, de Koning A P J, Pratt T S and Osuna R 2007 Effects of Fis on *Escherichia coli* gene expression during different growth stages *Microbiology* **153** 2922–40
- Breier A M and Cozzarelli N R 2004 Linear ordering and dynamic segregation of the bacterial chromosome *Proc. Natl Acad. Sci. USA* **101** 9175–76
- Buenemann M and Lenz P 2010 A geometrical model for DNA organization in bacteria *PLoS One* **5** e13806
- Burov S and Barkai E 2008 Fractional langevin equation: overdamped, underdamped, and critical behaviors *Phys. Rev. E* **78** 031112
- Butan C, Hartnell L M, Fenton A K, Bliss D, Sockett R E, Subramaniam S and Milne J L S 2011 Spiral architecture of the nucleoid in *Bdellovibrio bacteriovorus* *J. Bacteriol.* **193** 1341–50
- Cabrera J E, Cagliero C, Quan S, Squires C L and Jin D J 2009 Active transcription of rRNA operons condenses the nucleoid in *Escherichia coli*: examining the effect of transcription on nucleoid structure in the absence of transcription *J. Bacteriol.* **191** 4180–5
- Cho H, McManus H R, Dove S L and Bernhardt T G 2011 Nucleoid occlusion factor SlmA is a DNA-activated FtsZ polymerization antagonist *Proc. Natl Acad. Sci. USA* **108** 3773–8
- Condam S, Tejedor V, Voituriez R, Bnichou O and Klafter J 2008 Probing microscopic origins of confined subdiffusion by first-passage observables *Proc. Natl Acad. Sci. USA* **105** 5675–80
- Confalonieri F, Elie C, Nadal M, de La Tour C, Forterre P and Duguet M 1993 Reverse gyrase: a helicase-like domain and a type I topoisomerase in the same polypeptide *Proc. Natl Acad. Sci. USA* **90** 4753–7
- Cosgriff S, Chintakayala K, Chim Y T A, Chen X, Allen S, Lovering A L and Grainger D C 2010 Dimerization and DNA-dependent aggregation of the *Escherichia coli* nucleoid protein and chaperone CbpA *Mol. Microbiol.* **77** 1289–300
- Cunha S, Odijk T, Suleymanoglu E and Woldringh C L 2001 Isolation of the *Escherichia coli* nucleoid *Biochimie* **83** 149–54
- Cunha S, Woldringh C L and Odijk T 2005 Restricted diffusion of DNA segments within the isolated *Escherichia coli* nucleoid *J. Struct. Biol.* **150** 226–32
- Dame R T, Noom M C and Wuite G J L 2006 Bacterial chromatin organization by H-NS protein unravelled using dual DNA manipulation *Nature* **444** 387–90
- Dame R T, Kalmykova O J and Grainger D C 2011 Chromosomal macrodomains and associated proteins: implications for DNA organization and replication in gram negative bacteria *PLoS Genet.* **7** e1002123
- Daube S S, Bracha D, Buxboim A and Bar-Ziv R H 2010 Compartmentalization by directional gene expression *Proc. Natl Acad. Sci. USA* **107** 2836–41
- DeGennes P G 1976 Dynamics of entangled polymer solutions: I. The Rouse model *Macromolecules* **9** 587
- DeGennes P G 1979 *Scaling Concepts in Polymer Physics* (Ithaca, NY: Cornell University Press)
- Derman A I, Lim-Fong G and Pogliano J 2008 Intracellular mobility of plasmid DNA is limited by the parA family of partitioning systems *Mol. Microbiol.* **67** 935–46
- de Vries R 2010 DNA condensation in bacteria: Interplay between macromolecular crowding and nucleoid proteins *Biochimie* **92** 1715–21
- Diamant H and Andelman D 2000 Binding of molecules to DNA and other semiflexible polymers *Phys. Rev. E* **61** 6740–9
- Dillon S C and Dorman C J 2010 Bacterial nucleoid-associated proteins, nucleoid structure and gene expression *Nature Rev. Microbiol.* **8** 185–95
- Dolgushev M and Blumen A 2009 Dynamics of semiflexible treelike polymeric networks *J. Chem. Phys.* **131** 044905
- Edwards S F and Freed K F 1969 The entropy of a confined polymer: I. *J. Phys. A: Gen. Phys.* **2** 145
- Enright M B and Leitner D M 2005 Mass fractal dimension and the compactness of proteins *Phys. Rev. E* **71** 011912
- Espéli O and Boccard F 2006 Organization of the *Escherichia coli* chromosome into macrodomains and its possible functional implications *J. Struct. Biol.* **156** 304–10
- Espéli O, Mercier R and Boccard F 2008 DNA dynamics vary according to macrodomain topography in the *E. coli* chromosome *Mol. Microbiol.* **68** 1418–27
- Estevez-Torres A and Baigl D 2011 DNA compaction: fundamentals and applications *Soft Matter* **7** 6746–56
- Farago O and Kantor Y 2000 Elasticity of Gaussian and nearly Gaussian phantom networks *Phys. Rev. E* **62** 6094–102
- Fritsche M, Li S, Heermann D W and Wiggins P A 2011 A model for *Escherichia coli* chromosome packaging supports transcription factor-induced DNA domain formation *Nucl. Acids Res.* **40** 972–80
- Graessley W W 1980 Linear viscoelasticity in Gaussian networks *Macromolecules* **13** 372–6
- Grainger D C, Hurd D, Harrison M, Holdstock J and Busby S J 2005 Studies of the distribution of *Escherichia coli* cAMP-receptor protein and RNA polymerase along the *E. coli* chromosome *Proc. Natl Acad. Sci. USA* **102** 17693–8
- Grainger D C, Hurd D, Goldberg M D and Busby S J W 2006 Association of nucleoid proteins with coding and non-coding segments of the *Escherichia coli* genome *Nucl. Acids Res.* **34** 4642–52
- Grainger D C, Aiba H, Hurd D, Browning D F and Busby S J 2007 Transcription factor distribution in *Escherichia coli*: studies with FNR protein *Nucl. Acids Res.* **35** 269–78
- Grainger D C, Goldberg M D, Lee D J and Busby S J 2008 Selective repression by Fis and H-NS at the *Escherichia coli* dps promoter *Mol. Microbiol.* **68** 1366–77

- Grosberg A and Khokhlov A 1994 *Statistical Physics of Macromolecules* (New York: AIP)
- Grosberg A, Rabin Y, Havlin S and Neer A 1993 Crumpled globule model of the three-dimensional structure of DNA *Europhys. Lett.* **23** 373–8
- Helvoort J M V, Huls P G, Vischer N O and Woldringh C L 1998 Fused nucleoids re segregate faster than cell elongation in *Escherichia coli* pbpB(Ts) filaments after release from chloramphenicol inhibition *Microbiology* **144** 1309–17
- He Y, Burov S, Metzler R and Barkai E 2008 Random time-scale invariant diffusion and transport coefficients *Phys. Rev. Lett.* **101** 058101
- Huang W-H, Zinchenko A A, Pawlak C, Chen Y and Baigl D 2007 Dynamic conformational behavior and molecular interaction discrimination of DNA/binder complexes by single-chain stretching in a microdevice *ChemBiochem* **8** 1771–4
- Janssen H-K and Stenull O 2011 Collapse transition of randomly branched polymers: renormalized field theory *Phys. Rev. E* **83** 051126
- Jasch F, von Ferber Ch and Blumen A 2003 Dynamics of randomly branched polymers: configuration averages and solvable models *Phys. Rev. E* **68** 051106
- Jensen R B, Wang S C and Shapiro L 2001 A moving DNA replication factory in *Caulobacter crescentus* *EMBO J.* **20** 4952–63
- Jin D J and Cabrera J E 2006 Coupling the distribution of RNA polymerase to global gene regulation and the dynamic structure of the bacterial nucleoid in *Escherichia coli* *J. Struct. Biol.* **156** 284–91
- Joshi M C, Bourniquel A, Fisher J, Ho B T, Magnan D, Kleckner N and Bates D 2011 *Escherichia coli* sister chromosome separation includes an abrupt global transition with concomitant release of late-splitting intersister snaps *Proc. Natl Acad. Sci. USA* **108** 2765–70
- Jung Y and Ha B-Y 2010 Overlapping two self-avoiding polymers in a closed cylindrical pore: Implications for chromosome segregation in a bacterial cell *Phys. Rev. E* **82** 51926–31
- Junier I, Martin O and Képès F 2010 Spatial and topological organization of DNA chains induced by gene co-localization *PLoS Comput. Biol.* **6** e1000678
- Jun S and Wright A 2010 Entropy as the driver of chromosome segregation *Nature Rev. Micro.* **8** 600–7
- Jun S and Mulder B 2006 Entropy-driven spatial organization of highly confined polymers: lessons for the bacterial chromosome *Proc. Natl Acad. Sci. USA* **103** 12388–93
- Kahramanoglou C, Seshasayee A S N, Prieto A I, Ibberson D, Schmidt S, Zimmermann J, Benes V, Fraser G M and Luscombe N M 2011 Direct and indirect effects of H-NS and Fis on global gene expression control in *Escherichia coli* *Nucl. Acids Res.* **39** 2073–91
- Kantor Y and Kardar M 1996 Conformations of randomly linked polymers *Phys. Rev. E* **54** 5263–7
- Kavenoff R and Bowen B C 1976 Electron microscopy of membrane-free folded chromosomes from *Escherichia coli* *Chromosoma* **59** 89–101
- Képès F and Valliant C 2003 Transcription-based solenoidal model of chromosome *ComplexUs* **1** 171–80
- Kim J, Yoshimura S H, Hizume K, Ohniwa R L, Ishihama A and Takeyasu K 2004 Fundamental structural units of the *Escherichia coli* nucleoid revealed by atomic force microscopy *Nucl. Acids Res.* **32** 1982–92
- Kolatka K, Witosinska M, Pierechod M and Konieczny I 2008 Bacterial partitioning proteins affect the subcellular location of broad-host-range plasmid RK2 *Microbiology* **154** 2847–56
- Konopka M C, Shkel I A, Cayley S, Record M T and Weisshaar J C 2006 Crowding and confinement effects on protein diffusion *in vivo* *J. Bacteriol.* **188** 6115–23
- Lee C-Y 2006 Mass fractal dimension of the ribosome and implication of its dynamic characteristics *Phys. Rev. E* **73** 042901
- Lesterlin C, Mercier R, Boccard F, Barre F X and Cornet F 2005 Roles for replicohores and macrodomains in segregation of the *Escherichia coli* chromosome *EMBO Rep.* **6** 557–62
- Lieberman-Aiden E *et al* 2009 Comprehensive mapping of long-range interactions reveals folding principles of the human genome *Science* **326** 289–93
- Lim C J, Whang Y R, Kenney L J and Yan J 2011 Gene silencing H-NS paralogue StpA forms a rigid protein filament along DNA that blocks DNA accessibility *Nucl. Acids Res.* **40** 3316–28
- Liu X, Wang X, Reyes-Lamothe R and Sherratt D 2010 Replication-directed sister chromosome alignment in *Escherichia coli* *Mol. Microbiol.* **75** 1090–7
- Luijsterburg M S, Noom M C, Wuite G J and Dame R T 2006 The architectural role of nucleoid-associated proteins in the organization of bacterial chromatin: a molecular perspective *J. Struct. Biol.* **156** 262–72
- Marenduzzo D, Finan K and Cook P R 2006a The depletion attraction: an underappreciated force driving cellular organization *J. Cell. Biol.* **175** 681–6
- Marenduzzo D, Micheletti C and Cook P R 2006b Entropy-driven genome organization *Biophys. J.* **90** 3712–21
- Marenduzzo D, Micheletti C and Orlandini E 2010 Biopolymer organization upon confinement *J. Phys. Condens. Matter* **22** 283102
- Marko J F 2009 Linking topology of tethered polymer rings with applications to chromosome segregation and estimation of the knotting length *Phys. Rev. E* **79** 051905
- Marko J F and Cocco S 2003 The micromechanics of DNA *Phys. World* 37–41 <http://www.phys.ens.fr/~cocco/Art/24physworld.pdf>
- Marr C, Geertz M, Hütt M T and Muskhelishvili G 2008 Dissecting the logical types of network control in gene expression profiles *BMC Syst. Biol.* **2** 18
- Mateos-Langerak J *et al* 2009 Spatially confined folding of chromatin in the interphase nucleus *Proc. Natl Acad. Sci. USA* **106** 3812–7
- Mathelier A and Carbone A 2010 Chromosomal periodicity and positional networks of genes in *Escherichia coli* *Mol. Syst. Biol.* **6** 366
- Maurer S, Fritz J and Muskhelishvili G 2009 A systematic *in vitro* study of nucleoprotein complexes formed by bacterial nucleoid-associated proteins revealing novel types of DNA organization *J. Mol. Biol.* **387** 1261–76
- Meile J-C, Mercier R, Stouf M, Pages C, Bouet J-Y and Cornet F 2011 The terminal region of the *E. coli* chromosome localises at the periphery of the nucleoid *BMC Microbiol.* **11** 28
- Mercier R, Petit M-A, Schbath S, Robin S, Karoui M El, Boccard F and Espéli O 2008 The MatP/matS site-specific system organizes the terminus region of the *E. coli* chromosome into a macrodomain *Cell* **135** 475–85
- Mirny L A 2011 The fractal globule as a model of chromatin architecture in the cell *Chromosome Res.* **19** 37–51
- Moulin L, Rahmouni A R and Boccard F 2005 Topological insulators inhibit diffusion of transcription-induced positive supercoils in the chromosome of *Escherichia coli* *Mol. Microbiol.* **55** 601–10
- Muskhelishvili G, Sobetzko P, Geertz M and Berger M 2010 General organisational principles of the transcriptional regulation system: a tree or a circle? *Mol. Biosyst.* **6** 662–76
- Nicodemi M and Prisco A 2009 Thermodynamic pathways to genome spatial organization in the cell nucleus *Biophys. J.* **96** 2168–77
- Nielsen H J, Ottesen J R, Youngren B, Austin S J and Hansen F G 2006 The *Escherichia coli* chromosome is organized with the

- left and right chromosome arms in separate cell halves *Mol. Microbiol.* **62** 331–8
- Nielsen H J, Youngren B, Hansen F G and Austin S 2007 Dynamics of *Escherichia coli* chromosome segregation during multifork replication *J. Bacteriol.* **189** 8660–6
- Niki H, Yamaichi Y and Hiraga S 2000 Dynamic organization of chromosomal DNA in *Escherichia coli* *Genes Dev.* **14** 212–23
- Norris V 1995 Hypothesis: chromosome separation in *Escherichia coli* involves autocatalytic gene expression, transertion and membrane-domain formation *Mol. Microbiol.* **16** 1051–7
- Ochman H and Groisman E A 1994 The origin and evolution of species differences in *Escherichia coli* and salmonella typhimurium *EXS* **69** 479–93
- Odjik T and Ubbink J 1998 Dimensions of a plectonemic DNA supercoil under fairly general perturbations *Physica A* **252** 61–6
- Odjik T 1998 Osmotic compaction of supercoiled DNA into a bacterial nucleoid *Biophys. Chem.* **73** 23–9
- Ohniwa R L, Ushijima Y, Saito S and Morikawa K 2011 Proteomic analyses of nucleoid-associated proteins in *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* *PLoS One* **6** e19172
- Ohniwa R L, Morikawa K, Kim J, Ohta T, Ishihama A, Wada C and Takeyasu K 2006 Dynamic state of DNA topology is essential for genome condensation in bacteria *EMBO J.* **25** 5591–602
- Oshima T, Ishikawa S, Kurokawa K, Aiba H and Ogasawara N 2006 *Escherichia coli* histone-like protein H-NS preferentially binds to horizontally acquired DNA in association with RNA polymerase *DNA Res.* **13** 141–53
- Pitard E and Orland H 1998 Dynamics of the swelling or collapse of a homopolymer *Europhys. Lett.* **41** 467–72
- Postow L, Hardy C D, Arsuaga J and Cozzarelli N R 2004 Topological domain structure of the *Escherichia coli* chromosome *Genes Dev.* **18** 1766–79
- Reuveni S, Granek R and Klafter J 2010 Anomalies in the vibrational dynamics of proteins are a consequence of fractal-like structure *Proc. Natl Acad. Sci. USA* **107** 13696–700
- Reyes-Lamothe R, Possoz C, Danilova O and Sherratt D J 2008 Independent positioning and action of *Escherichia coli* replisomes in live cells *Cell* **133** 90–102
- Robinow C and Kellenberger E 1994 The bacterial nucleoid revisited *Microbiol. Rev.* **58** 211–32
- Rocha E P C 2008 The organization of the bacterial genome *Annu. Rev. Genet.* **42** 211–33
- Romantsov T, Fishov I and Krichevsky O 2007 Internal structure and dynamics of isolated *Escherichia coli* nucleoids assessed by fluorescence correlation spectroscopy *Biophys. J.* **92** 2875–84
- Sánchez-Romero M A, Busby S J W, Dyer N P, Ott S, Millard A D and Grainger D C 2010 Dynamic distribution of SeqA protein across the chromosome of *Escherichia coli* K-12 *MBio* **1** e0012-1
- Schneider R, Lurz R, Lder G, Tolksdorf C, Travers A and Muskhelishvili G 2001 An architectural role of the *Escherichia coli* chromatin protein FIS in organising DNA *Nucl. Acids Res.* **29** 5107–14
- Scolari V F, Bassetti B, Sclavi B and Lagomarsino M C 2011 Gene clusters reflecting macrodomain structure respond to nucleoid perturbations *Mol. Biosyst.* **7** 878–88
- Scott M and Hwa T 2011 Bacterial growth laws and their applications *Curr. Opin. Biotechnol.* **22** 559–65
- Scott M, Gunderson C W, Mateescu E M, Zhang Z and Hwa T 2010 Interdependence of cell growth and gene expression: origins and consequences *Science* **330** 1099–102
- Sherratt D J 2003 Bacterial chromosome dynamics *Science* **301** 780–5
- Shopland L S *et al* 2006 Folding and organization of a contiguous chromosome region according to the gene distribution pattern in primary genomic sequence *J. Cell. Biol.* **174** 27–38
- Skoko D, Yoo D, Bai H, Schnurr B, Yan J, McLeod S M, Marko J F and Johnson R C 2006 Mechanism of chromosome compaction and looping by the *Escherichia coli* nucleoid protein fis *J. Mol. Biol.* **364** 777–98
- Sommer J U and Blumen A 1995 On the statistics of generalized Gaussian structures: collapse and random external fields *J. Phys. A: Math. Gen.* **28** 6669
- Stavans J and Oppenheim A 2006 DNA-protein interactions and bacterial chromosome architecture *Phys. Biol.* **3** R1–10
- Stuger R, Woldringh C L, van der Weijden C C, Vischer N O E, Bakker B M, van Spanning R J M, Snoep J L and Weterhoff H V 2002 DNA supercoiling by gyrase is linked to nucleoid compaction *Mol. Biol. Rep.* **29** 79–82
- Sumedha and Weigt M 2008 A thermodynamic model for the agglomeration of DNA-looping proteins *J. Stat. Mech. Theory Exp.* **2008** P11005
- Thanbichler M and Shapiro L 2006 Chromosome organization and segregation in bacteria *J. Struct. Biol.* **156** 292–303
- Thanbichler M, Wang S C and Shapiro L 2005 The bacterial nucleoid: a highly organized and dynamic structure *J. Cell. Biochem.* **96** 506–21
- Tonthat N Ky, Arold S T, Pickering B F, Dyke M W V, Liang S, Lu Y, Beuria T K, Margolin W and Schumacher M A 2011 Molecular mechanism by which the nucleoid occlusion factor, SlmA, keeps cytokinesis in check *EMBO J.* **30** 154–64
- Toro E and Shapiro L 2010 Bacterial chromosome organization and segregation *Cold Spring Harb Perspect Biol.* **2** a000349
- Trun N J and Marko J F 1998 Architecture of a bacterial chromosome (review) *Am. Soc. Microbiol. News* **64** 276
- Ubbink J and Odjik T 1999 Electrostatic-undulatory theory of plectonemically supercoiled DNA *Biophys. J.* **76** 2502–19
- Umbarger M A *et al* 2011 The three-dimensional architecture of a bacterial genome and its alteration by genetic perturbation *Mol. Cell.* **44** 252–64
- Valens M, Penaud S, Rossignol M, Cornet F and Boccard F 2004 Macrodomain organization of the *Escherichia coli* chromosome *EMBO J.* **23** 4330–41
- van Helvoort J M, Kool J and Woldringh C L 1996 Chloramphenicol causes fusion of separated nucleoids in *Escherichia coli* K-12 cells and filaments *J. Bacteriol.* **178** 4289–93
- van Noort J, Verbrugge S, Goosen N, Dekker C and Dame R T 2004 Dual architectural roles of HU: formation of flexible hinges and rigid filaments *Proc. Natl Acad. Sci. USA* **101** 6969–74
- Vettorel T, Grosberg A Y and Kremer K 2009 Statistics of polymer rings in the melt: a numerical simulation study *Phys. Biol.* **6** 025013
- Viollier P H, Thanbichler M, McGrath P T, West L, Meewan M, McAdams H H and Shapiro L 2004 Rapid and sequential movement of individual chromosomal loci to specific subcellular locations during bacterial DNA replication *Proc. Natl Acad. Sci. USA* **101** 9257–62
- Vora T, Hottes A K and Tavazoie S 2009 Protein occupancy landscape of a bacterial genome *Molecular Cell* **35** 247–53
- Wade J T, Struhl K, Busby S J and Grainger D C 2007 Genomic analysis of protein-DNA interactions in bacteria: insights into transcription and chromosome organization *Mol. Microbiol.* **65** 21–6
- Wang W, Li G-W, Chen C, Xie X S and Zhuang X 2011a Chromosome organization by a nucleoid-associated protein in live bacteria *Science* **333** 1445–9
- Wang X, Lesterlin C, Reyes-Lamothe R, Ball G and Sherratt D J 2011b Replication and segregation of an *Escherichia coli* chromosome with two replication origins *Proc. Natl Acad. Sci. USA* **108** E243–50
- Wang X, Liu X, Possoz C and Sherratt D J 2006 The two *Escherichia coli* chromosome arms locate to separate cell halves *Genes Dev.* **20** 1727–31
- Warren P B and ten Wolde P R 2004 Statistical analysis of the spatial distribution of operons in the transcriptional

- regulation network of *Escherichia coli* *J. Mol. Biol.* **342** 1379–90
- Weber S C, Spakowitz A J and Theriot J A 2010a Bacterial chromosomal loci move subdiffusively through a viscoelastic cytoplasm *Phys. Rev. Lett.* **104** 238102
- Weber S C, Theriot J A and Spakowitz A J 2010b Subdiffusive motion of a polymer composed of subdiffusive monomers *Phys. Rev. E* **82** 011913
- Wiese K J 1998 Dynamics of selfavoiding tethered membranes I Model A Dynamics (Rouse Model) *Eur. Phys. J.* **B1** 269–72
- Wiggins P A, Cheveralls K C, Martin J S, Lintner R and Kondev J 2010 Strong intranucleoid interactions organize the *Escherichia coli* chromosome into a nucleoid filament *Proc. Natl Acad. Sci.* **107** 4991–5
- Wiggins P A, Dame R Th, Noom M C and Wuite G J L 2009 Protein-mediated molecular bridging: a key mechanism in biopolymer organization *Biophys. J.* **97** 1997–2003
- Woldringh C L and Nanninga N 2006 Structural and physical aspects of bacterial chromosome segregation *J. Struct. Biol.* **156** 273–83
- Xu W and Muller S J 2012 Polymer-monovalent salt-induced DNA compaction studied via single-molecule microfluidic trapping *Lab Chip* **12** 647–51
- Zaslaver A, Kaplan S, Bren A, Jinich A, Mayo A, Dekel E, Alon U and Itzkovitz S 2009 Invariant distribution of promoter activities in *Escherichia coli* *PLoS Comput. Biol.* **5** e1000545
- Zhang C, Shao P G, van Kan J A and van der Maarel J R C 2009 Macromolecular crowding induced elongation and compaction of single DNA molecules confined in a nanochannel *Proc. Natl Acad. Sci. USA* **106** 16651–6
- Zimmerman S B 2004 Studies on the compaction of isolated nucleoids from *Escherichia coli* *J. Struct. Biol.* **147** 146–58
- Zimmerman S B 2006a Cooperative transitions of isolated *Escherichia coli* nucleoids: implications for the nucleoid as a cellular phase *J. Struct. Biol.* **153** 160–75
- Zimmerman S B 2006b, Shape and compaction of *Escherichia coli* nucleoids *J. Struct. Biol.* **156** 255–61
- Zimm B H and Stockmayer W H 1949 The dimensions of chain molecules containing branches and rings *J. Chem. Phys.* **17** 1301