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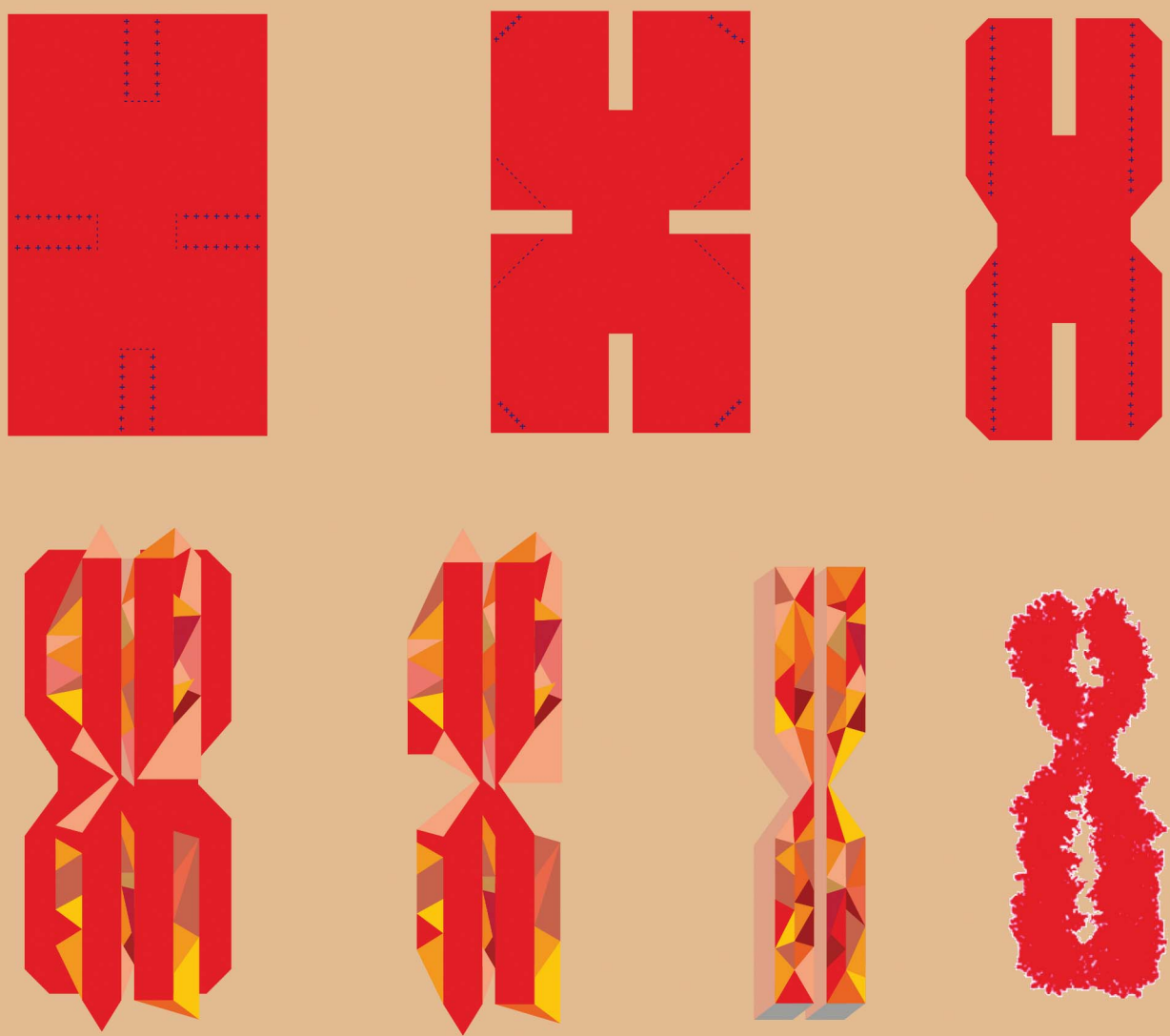

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REVIEW ARTICLE

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REVIEW

Electrostatics of DNA compaction in viruses, bacteria and eukaryotes: functional insights and evolutionary perspective

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The molecular support of genetic information, DNA, has to be packaged and organized inside the tiny volume of nuclei, cells, or virus capsids, in an ordered and dynamical way. Evolution has favored different strategies in different kingdoms: a liquid crystal ordering mechanism prevails in viruses; strong entanglement due to supercoiling is the main compacting strategy in bacteria; the building of a hierarchical and tunable architecture mediated by DNA–protein interaction constitutes the main compacting mechanism in archaea and eukaryotes. The interplay between these different strategies is however much more complex than at first sight and all these strategies can be used in a synergistic way. All of them have to deal with the same elementary physical constraint which hinders compaction: electrostatic repulsion due to the high line charge density of DNA. In this review, we will show how this apparent weakness can be turned into a strength in order to compact this long molecule in a functional way.

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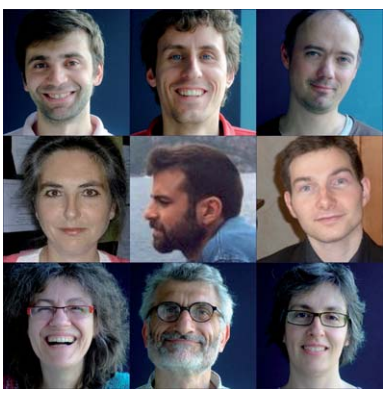

Pascal Carrivain, **Axel Cournac**, **Christophe Lavelle**, **Annick Lesne**, **Julien Mozziconacci**, **Fabien Paillusson**, **Laurence Signon**, **Jean-Marc Victor**, **Maria Barbi**

This review is a collective work of the "Multiscale Modeling of Living Matter" group (http://www.lptl.jussieu.fr/user/pipo/M3V/m3v.html). The group activity is devoted to the physical modeling of chromosome architecture and functional dynamics, from the atomic scale up to the whole nucleus, with a special focus on protein–DNA interaction, nucleosome plasticity and chromatin fiber structures. The group was founded in 2000 by Annick Lesne and Jean-Marc Victor, both CNRS Directors of Research in physics. Maria Barbi is a DNA physicist; she joined the group in 2001 as an associate professor. Julien Mozziconacci was a PhD student in the group from 2001 to 2004; he joined the group as an associate professor in 2009. Christophe Lavelle was an assistant lecturer in the group from 2002 to 2003. He was appointed CNRS Research Associate in 2008 at the "Museum National d'Histoire Naturelle" in Paris. Laurence Signon is a DNA repair biologist, CNRS Research Associate since 2000; she joined the group in 2011 for a two year period to learn physical modeling techniques. Fabien Paillusson was a PhD student in the group from 2007 to 2010; he is now a postdoctoral researcher in Daan Frenkel's group in Cambridge. Axel Cournac joined the group as a postdoctoral researcher in 2011. Pascal Carrivain is a PhD student in the group since 2009.

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1 Introduction

Genetic information is encoded essentially in the DNA double helix. The genetic material has to be packaged inside the tiny volume of the cell, and specific sequences have to be retrieved at will for physiological purposes. The genetic material is therefore embedded in an orderly and dynamically retrievable architecture. Knowledge of the physical principles and the molecular machinery that govern the 3D organization of this structure and its regulation is key to understanding the relationship between genome structure and function. DNA is one of the most highly charged polyelectrolytes, and also one of the stiffest polymers. How both these physical constraints have been overcome and actually turned to good account during evolution of living organisms is the focus of this review.

If DNA is the genetic support for all living organisms, the way it is organized in the available space may differ considerably from viruses to prokaryotes (organisms that lack cell nucleus, generally unicellular, as bacteria), to archaea (again nucleus-lacking unicellular organisms, but having an independent evolutionary history), to eukaryotes (organisms having a cell nucleus, including multicellular organisms). Different organisms achieve different DNA compaction levels. To quantify this compaction level, one can define two different quantities (see Table 1). As shown in Table 1, the various biological compaction strategies implemented among the different organisms cannot be reduced to the coil–globule transition of a polyelectrolyte but involve instead architectural proteins that constitute the very folds of a "molecular origami".

The paper is organized as follows: in Section 2, we present the molecular actors (DNA, ions, water, proteins) and describe the elementary electrostatics features involved in their interaction. In Section 3, we discuss how electrostatics may drive three fundamental mechanisms involved in genome condensation, namely DNA supercoiling, the building of an architecture by DNA binding proteins (BPs) and liquid crystal ordering of DNA and proteins. The way how different condensation mechanisms are implemented *in vivo* in different kingdoms (viruses, prokaryotes, eukaryotes and archaea) is then discussed in Section 4. Section 5 is devoted to deeper insights into the functional tuning allowing regulation of DNA compaction, again focusing on the role of electrostatics. Finally, in Section 6 we reexamine the overall scenario from an evolutionary perspective, and draw a few conclusions.

2 DNA, proteins, ions and their electrostatic interactions

2.1 DNA in solution and Manning condensation

Double stranded DNA (dsDNA) is a highly charged polyelectrolyte of $-2e$ per base pair, each elementary charge belonging to one backbone phosphate. It is also one of the stiffest polymers in nature. The high charge, together with the cylinder-like shape of DNA, induces the formation of a condensed counterion atmosphere around the dsDNA "cylinder". In the 70s, Manning introduced a theoretical approach to calculate the condensed counterion density,[131] and showed that condensation depends on the value of the Manning parameter $\xi = q\lambda l_B$, where q is the counterion valency, λ the effective line charge density of the rod and $l_B \simeq 7$ Å the Bjerrum length in normal temperature conditions (the Bjerrum length is defined as the separation at which the electrostatic interaction between two elementary charges is comparable in magnitude to the thermal energy scale, k_BT). If $\xi < 1$, the bare charge on DNA is not enough to overcome the entropic cost of binding for counterions and they escape from the polyelectrolyte whereas if $\xi > 1$ a counterion condensation is predicted and the rod line charge density is expected to be renormalized as $\lambda_{eff} = (ql_B)^{-1}$. In the case of a monovalent salt, Manning theory predicts that, due to the high DNA charge, the number of condensed monovalent counterions is 0.76 per DNA phosphate charge, independent of the added salt concentration. The counterion density around DNA is therefore of about 3 positive charges every 2 base pairs, this resulting in a linear charge of $-e$ every 0.68 nm or, alternatively, in an average surface charge $\sigma_{DNA} = -e$ nm^{-2}. Note that temperature may vary considerably from one organism to another. This will change in turn the Bjerrum length and the Manning parameter ξ accordingly. Different strategies will therefore be used in different organisms to deal with DNA electrostatics.

As mentioned above, the Manning parameter ξ grows linearly with the valency of the counterions so that multivalent ions more easily condense on DNA. Despite the robustness of its general features, it has been stressed that Manning condensation depends on the chemical nature of the counterions, even for monovalent ions.[30,85,200]

This is likely due to the way these ions interact with the solvent. Small monovalent ions of high charge density (as Li^+ or Na^+) bind water molecules strongly, whereas large monovalent ions of low charge density (as K^+ or Cs^+, but also arginine, histidine, and lysine side chains) bind water molecules weakly relative to the strength of water–water interactions in bulk solution.[36] This results in a difference in the ion solubility, and therefore in their adsorption on macromolecular surfaces.[36]

2.2 Mechanics of DNA in solution

Electrostatics of DNA in solution affects its mechanical properties. Three key parameters of the DNA double helix come

Table 1 Genome compaction for different organisms. The volume reduction is evaluated, following Holmes and Cozzarelli,[88] as the ratio between the volume occupied by a DNA stretch of equivalent length free in solution and the volume of the container (capsid, cell or nucleus). The packing fraction is the ratio between the physical volume of DNA and the volume of the container, where the physical volume of DNA is defined as the volume of a cylinder of same length as the whole genome and radius equal to 1 nm

Organism	Sequence length (bp)	Volume reduction	Packing fraction (%)
E. coli	4.6×10^6	3.7×10^2 (ref. 88)	1.2
Yeast	2.8×10^7	1.9×10^3 (ref. 88)	0.7
Human	6.0×10^9	4.9×10^4 (ref. 88)	1.2
Virus (T4)	0.17×10^6	6.9×10^3 (ref. 15)	67

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into play: both bending and torsional persistence lengths and the effective diameter. The bending persistence length is defined as the length over which correlations in the direction of the tangent to the polymer vanish. Its large value is due, in part, to the strongly charged polyelectrolyte nature of the DNA: repelling each other, these charges act against the double helix bending. On the other hand, the stacking interaction between DNA base pairs also contribute to the stability of the double helix and hence to its rigidity. By charging the DNA phosphate groups from a valency of zero to their normal valency of −1, the simulation by Savelyev *et al.* showed that the electrostatic contribution to the persistence length is roughly equal to the contribution from base stacking and other possible non-electrostatic sources.[201] The twisting persistence length is similarly defined and accounts for torsional rigidity, while the effective diameter accounts for electrostatic repulsion between two dsDNAs. These parameters determine how mechanical constraints are transmitted along DNA and transducted into compaction or decompaction.

DNA mechanical properties have been measured most efficiently through single DNA molecule manipulation by optical or magnetic tweezers. Indeed, such experiments give a direct access to the mechanical response of DNA under a stretching force and torsional constraints. Both bending and twisting rigidities as well as the effective radius of DNA have thus been probed in various salt conditions.[160] These experiments together with former measurements (light scattering or sedimentation) have shown that the bending persistence length slowly decreases from ∼70 nm down to ∼30 nm while increasing monovalent salt concentration for over three decades (from 2.5 mM up to 3 M).[81,202] Whereas it used to be assumed that the bending persistence length reaches a lower limit[81] (close to 50 nm) above ∼20 mM, several authors recently started to critically reexamine this assumption by theoretical[132] and computational[202] means and came to the conclusion that the bending persistence length steadily drops down with increasing salt concentration (down to ∼30 nm at 1 M). In the presence of multivalent ions at mM concentrations, the bending persistence length is strongly reduced and can reach 25 nm.[134] Again, the ion type matters, and ions in which the charge is centrally concentrated (point-like cations as Mg^{2+} or $Co(NH_3)_6^{3+}$) yield lower values than ions in which the charge is linearly distributed (mainly polyamines, *e.g.* spermidine^{3+} or spermine^{4+}).[10]

As for the twisting persistence length, it is hardly affected by the monovalent salt whereas it was shown to decrease[160] from ∼100 nm to ∼60 nm when adding 5 mM Mg^{2+} into the solution. This change in persistence length can be accompanied by a change in the DNA local structure.[191]

Finally, many papers[20,192,193,215] give an estimation of the effective DNA diameter for different salt concentrations and show that it decreases from 15 to approximately 2 nm while increasing the monovalent salt concentration from 0.01 to 1 M. Divalent ions, such as Mg^{2+} even at mM concentrations, also strongly reduce the effective diameter down to less than 5 nm and therefore close to its crystallographic value.[192,193]

All these salt effects are summed up in a schematic representation in Fig. 1.

2.3 DNA–DNA ion-mediated interaction

Ion mediated interactions have been shown experimentally and numerically to induce an attractive interaction between like charged objects such as dsDNAs.[8,79,89,96,121,133,147,155,235,237] An ion mediated attraction between like charged objects is not catchable within the well known Poisson–Boltzmann (PB) theory[158,159,171,194] and hence other approaches have been proposed during the last 15 years. The extent of the attraction as well as the possible physical mechanisms responsible for it are, however, counterion-type dependent. In the following three paragraphs we review the three main contributions to this topic.

(i) Nanoscale cluster formation and measurements of virial coefficients incompatible with purely repulsive ion mediated interactions have been observed in dsDNA solutions with monovalent salt only (reviewed in ref. 133). These effects are all the more strong that the ionic strength is low. To explain these observations, Manning has suggested an extension of its condensation theory involving two counterion populations: condensed counterions and bulk counterions. Such a description leads to an attraction between two dsDNAs at a finite distance. The underlying mechanism is akin to covalent bonding in the sense that the sharing of condensed counterions by two close dsDNAs results in an increase in their translational entropy.[133,180] In this respect, the original paper by Ray and Manning is enlightening.[180]

(ii) Multivalent ions with a centrally concentrated charge greater than +3 (*e.g.* Cr^{3+}) or specific divalent metal ions such as Co^{2+} and Cd^{2+} are known to lead to dsDNA aggregation, while usual divalent ions (*e.g.* Mg^{2+}) are able to do so only with triple-stranded DNAs.[179] Theoretical strategies to account for these observations are twofold. On one hand, one can try to devise an effective model of the charge pattern on a *counterion dressed* dsDNA that would lead to an attraction within the PB theory. On the other hand, a theoretical treatment of the ion statistics that includes correlations can be proposed. An example of the first category is the so-called Kornyshev–Leikin (KL) theory which assumes that the charge pattern on dsDNA does not comprise negative charges only but also carries a fraction of irreversibly adsorbed cations mostly in the major groove.[38,107–109] This strong assumption is not discussed at the chemical level in the theory and a strict interpretation of this model might be contradictory with some experiments done on divalent metal ions for instance.[99–102] Despite this issue, one can compute the interaction between such dressed dsDNAs and predict an exponentially decaying attraction between two side by side dsDNA segments with a decay length that is almost salt independent, $\lambda_{KL} \simeq H/2\pi \simeq 5$ Å (where $H \approx 3.4$ nm is the DNA average helical pitch). This attraction originates from the dipole-moment distribution on each dsDNA segment. The electrostatic attraction is predicted to be maximum for two homologous dsDNAs and when one of the dsDNAs is shifted along its axis by half its average helical pitch, an ideal "electrostatic zipper" is formed, as shown schematically in Fig. 2. If the dsDNA molecules are not homologous, this approach still predicts an attraction, but of much weaker intensity.[38,107,109] The simplest attempt for the second category of strategies considers bare dsDNA molecules carrying only negative charges in solution and tries to account for very strong ion–ion and ion–DNA correlations in the system.

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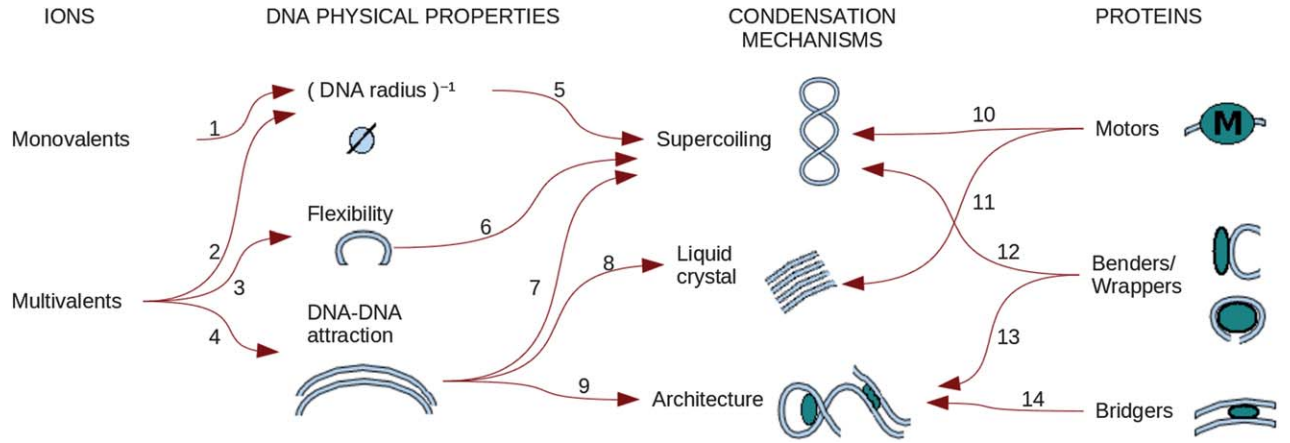

Fig. 1 Three main condensation mechanisms of DNA and the different impacts of ions, DNA physical properties and proteins are shown. Each arrow with its number corresponds to a precise point that is explained in a section of the text (arrows 1–3: Section 2.2, arrow 4: Section 2.3, arrows 5–7: Section 3.1, arrow 8: Section 3.4, arrow 9: Section 3.2, arrow 10: Section 3.1, arrow 11: Section 4.1, arrows 12–14: Section 3.2).

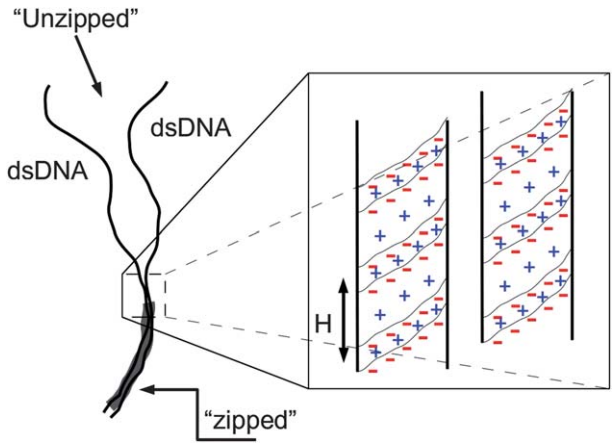

Fig. 2 Schematic representation of the DNA–DNA interaction mechanism leading to attraction. If two double stranded DNA molecules with positive counterions irreversibly adsorbed in the major and minor grooves come in close contact with the appropriate phase shift, a "zipper"-like mechanism could happen between facing opposite charges.

This is the so-called Strong Coupling (SC) regime, where the ion–DNA correlations are so strong that they lead to the condensation of most counterions in an almost two-dimensional strongly correlated liquid at the surface of the DNA. In the case where the two-dimensional liquid is so correlated that it tends to form a two-dimensional Wigner crystal, the charge pattern at the surface of the DNA (that is comprised of adsorbed positive counterions on negative patches) displays a dipole-moment structure that leads to an exponentially decaying attraction akin to the "electrostatic zipper" of the KL theory.[115,116,190,207] This Wigner crystal picture has got a regain of interest recently and has been shown to give very good agreement with Monte Carlo simulations.[195,196] The SC regime has also been interpreted as a virial expansion whose first order of approximation would yield a single-counterion picture that also predicts a like charge attraction.[97,147,157] Even though its predictions diverge from MC simulation at higher orders for two plates,[195,196] the virial expansion has been quite successfully applied to uniformly charged,[96,155,156] and helically charged[95] cylinders. In particular, an attraction owing to ion–DNA electrostatic correlations is found to appear only for counterion valencies higher than +3 for parameters corresponding to dsDNAs in solution.

Recent experiments on condensed dsDNA arrays[47,228] have shown that the DNA–DNA interaction displays exponentially decaying repulsion and attraction with typical length scales that are not very sensitive neither to the charge of the condensing agents nor to their chemistry (the measured range for the attraction is about 5 Å). As stressed by De Rouchey *et al.* 2010,[47] the attraction is quite likely due to some positive correlation between complementary charge motifs that is present in both KL and SC theories. The "electrostatic zipper" picture seems therefore a fair representation of a plausible mechanism for DNA–DNA aggregation in the presence of charged and compact condensing agents.

(iii) Most of the charged condensing agents consist in a repetition of a positive rod-like molecule into multivalent chains (*e.g.* polyamines)[17,47,107,143,228] and this particular structure could be responsible for an attraction mechanism independent of ion–ion electrostatic correlation. In fact, it has been shown by Bohinc and co-workers[17,143] that rod-like divalent ions (two charges separated by a fixed distance) could lead to attraction between two like charged plates when their charge is treated within a mean field theory. When the distance between the plates is about the size of the rod, then it is electrostatically favorable for counterions to form bridges lying perpendicularly to the macromolecules. This effect is better illustrated by imagining a rod ion exactly at the midplane between two plates: the rod will be unstable if oriented parallel to the planes, while it will be stabilized in the perpendicular direction. Finally, if the size of the divalent rods is above a certain threshold, then this "bridging" transition may appear at distances where the electrostatic gain overcomes the electrostatic cost of bringing two like charged particles closer and this yields attraction. Further developments accounting for correlations and multivalent chains have been proposed recently by the same authors.[18]

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Overall, whatever the ion type, an attraction induced by counterions always relies on our current understanding of a *bridging* mechanism. This mechanism, as we have seen, can be due to an entropy increase induced by the sharing of condensed counterions, or a strong ion–macromolecule electrostatic correlation in the presence of high valency counterions, characterized either by a strongly localized charge, or by a smaller charge evenly distributed on a chain.

2.4 Electrostatics of the protein–DNA interaction

DNA-binding proteins are multivalent macroions that display charged patches which can bind to DNA.[93,167] Their shape and charge distribution determine their binding mode of interaction with the DNA double helix. Not surprisingly, patches are generally positively charged but some enzymes bear negatively charged patches, *e.g.* DNase I. Their binding to DNA is controlled by multivalent cations,[78] in a similar way as DNA–DNA ion-mediated attraction (see Section 2.3 above). Interestingly, proteins that recognize and bind to specific DNA sequences, such as transcription factors and restriction enzymes, seem to be on average less charged than non-specific DNA-BPs such as histones or nucleoid-associated proteins (NAPs, see Section 4.2). A possible reason for the lower charge observed for specific DNA-BPs is that it may play a role in facilitating protein sliding along DNA – a necessary step for finding target sequences. Indeed, these proteins have a characteristic concave shape, inducing a large fitting interface with DNA (of the order of a few tens of nm^2).[94] For the intermediate values of the protein charge, local electroneutrality imposes the trapping of compensating ions between the DNA and the charged patches of the protein facing the DNA. When the protein approaches DNA, the increasing ion density induces a relevant osmotic pressure. The consequent repulsion is able to push the protein far from the DNA surface, thus preventing the formation of chemical bonds: the protein mobility is increased and the sliding becomes possible.[42,167] Simple models predict that the larger the charge of the patch, the lower the number of ions trapped and the lower the equilibrium distance between the protein and the DNA.[42,166] Therefore, it is expected that highly charged DNA-BPs will strongly stick to DNA. On the contrary, specific DNA-BPs undergo stronger repulsion which is able to push the protein away from the DNA surface, far enough to prevent the formation of chemical bonds: the protein mobility is then increased and the sliding becomes possible.[42,167]

3 Mechanisms of DNA compaction

DNA, ions and proteins interact, in different organisms, as to built and organize the genome in a functional way. Electrostatics plays a crucial role in such interaction, and may justify by itself the simplest observed architectures, *e.g.* that observed in viruses. However, increasing complexity of the organisms needs an increasing involvement of specific structural features of proteins, then protein–protein interactions, and finally the emergence of a collective behavior should be invoked. The overall organization obtained is active, ATP dependent. Alternative architectures exist in most cases.

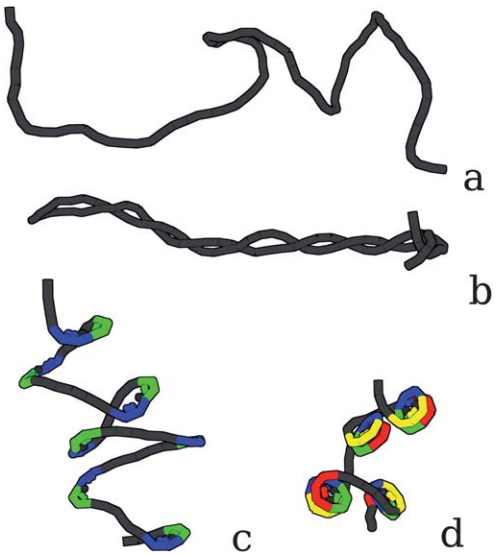

Fig. 3 Schematic view of two different mechanisms for DNA compaction: an unconstrained supercoiling and a constrained, protein-mediated architecture. A 900 bp long DNA is represented in a thermally disordered configuration (a) and in a supercoiled state (b). The same length of DNA is then organized by the formation of 7 tetrasomes (c) or 4 nucleosomes (d). Color code: blue and green spheres represent H3–H4 dimers or, equivalently, HMfA and HMfB archaeal proteins. Red and yellow spheres represent H2A–H2B dimers. DNA stretches that are bound to proteins are represented in the same color as the corresponding proteins. The four pictures are represented at the same scale.

Nonetheless, mechanics, electrostatics and ion-mediated effects still play a crucial role in tuning the dynamical evolution of such complex structures. We can identify three major fundamental mechanisms involved in genome condensation: (i) DNA over- or under-winding, *i.e.* DNA supercoiling (Fig. 3b), (ii) building of a (hierarchical) architecture supported by DNA folds mediated by proteins (Fig. 3c and d), and (iii) DNA–DNA aggregation inducing liquid crystal ordering, a mechanism that depends not only on the presence of multivalent ions but also on osmotic stress (Fig. 4). The interplay between these mechanisms may be sophisticated, and results in a wealth of possibilities. For example, supercoiling may accompany and enhance DNA–DNA interaction by braiding two dsDNAs.

In this section, we will discuss some details of these three fundamental mechanisms, while the *in vivo* implementation of these mechanisms will be discussed in Section 4. As a guide, Fig. 3 presents a schematic view of the first two compaction modes mentioned above.

3.1 DNA compaction and supercoiling

One way to compact DNA is to apply torsional constraints on it so as to achieve supercoiling. Torsional constraints applied on a rope produce either plectonemic or toroidal structures depending on the superhelical density (*i.e.* the number of turns that have been applied on the relaxed rope, divided by the length of the rope).[90] Plectonemes are loops of dsDNA helices twisted together, as commonly observed in garden hoses and telephone or computer cords, whereas toroids are annular-shaped

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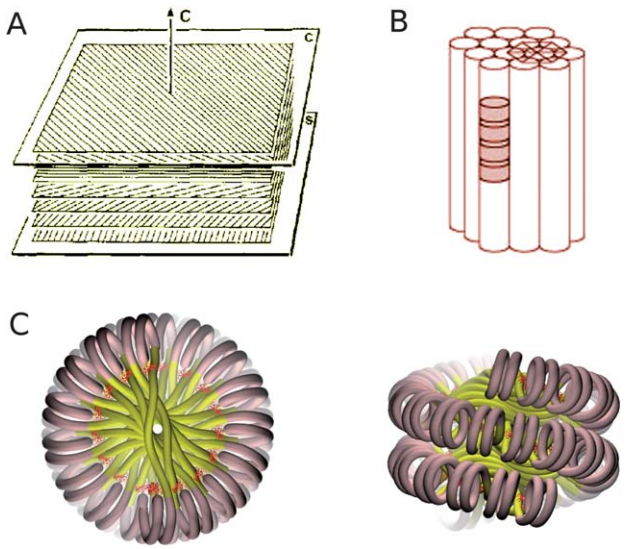

Fig. 4 Liquid-crystal order of DNA and nucleosomes. (A) Scheme of a DNA cholesteric right handed liquid crystal order. In each plane perpendicular to the cholesteric axis *C*, DNA molecules are arranged in the same orientation. The orientation in a particular plane is obtained from the orientation of DNA in the previous one by a rotation of an angle θ. The same orientation is therefore recovered every π/θ plane. Adapted from Livolant 1991.[122] (B) Stacks of nucleosomes in a nucleosome liquid crystalline order. Nucleosomes are represented as pink cylinders and columns of nucleosomes are shown to interact laterally. Adapted from Mangenot *et al.* 2003.[130] (C) Left and right: respectively top and side view of the chromatin fiber. Nucleosomes (in pink) are stacked in columns as in (B) and the DNA linkers (in yellow) are found in a similar cholesteric order as in (A). Linker histones H1 are shown in red.

structures favored at higher superhelical density. The critical value of the twisting torque necessary to induce plectonemes is called the buckling torque. Both DNA bending and twisting persistence lengths affect the buckling torque, together with another parameter, the DNA effective diameter, directly related to DNA–DNA electrostatic repulsion (see Section 2.2 above). Using magnetic tweezers,[187] Mosconi and co-workers[149] provided an experimental study of DNA supercoiling by applying a rotation to the magnetic bead (magnetic tweezers cannot impose a torque to the magnetic bead) at one DNA extremity while keeping the other clamped, at constant stretching force. A theoretical treatment[250] provides an indirect measurement of the critical buckling torque at different salt concentrations. Moreover, the buckling transition becomes steeper at higher salt concentrations, and disappears in low salt concentration solutions.[23,63] At low stretching forces (<0.3 pN) and physiological salt concentration (~200 mM) the buckling torque is estimated to be less than 4 pN nm (*i.e.* of the order of k_BT).[149] This suggests a relative ease in forming and modifying plectonemes *in vivo*. Interestingly, the buckling torque is much affected by the ionic strength, decreasing down to half its maximal (*i.e.* at low salt) value with increasing monovalent salt concentration, and rather independently from the stretching force value.

Adding multivalent cations to the solution is another way to induce supercoiling in DNA, and this results in well-defined condensed structures, particularly toroids and bundles of rods[14,53,58,59,140] similar to those observed in viruses (see Section 4.1). In this case, the zipping mechanism discussed in Section 2.3 is directly involved. However, multivalent cations also affect the formation of plectonemic structures. The plectoneme radius (also called supercoiling radius) ρ depends indeed on the concentration of the monovalent salt[234] and on the stretching force f too (ρ decreases when increasing f).[32,33,160,161] Interestingly, divalent ions, *e.g.* Mg^{2+}, strongly lower the supercoiling radius, thus facilitating plectoneme formation (actually by reducing the critical torque), which in turn facilitates bridging of both dsDNAs in the plectoneme. Moreover, at the atomistic level, Timsit and Varnai have shown that Mg^{2+} (and other divalent cations too) specifically promotes the formation of stable right-handed crossovers, so that DNA is preferentially condensed into left-handed plectonemes (hence positively supercoiled).[227]

3.2 Architectural proteins

Highly charged macroions can induce extensive bending and even wrapping of DNA. This effect can be explained by the electrostatic interactions between a charged flexible cylinder, modeling DNA, and a positively charged sphere.[4,205] In this model, the number of turns of DNA is determined, in physiological conditions, by the sphere charge and radius. This effect can obviously be extended to larger charged bodies, and in particular to proteins or protein complexes.

Interestingly, many of the known structural proteins involved in genome compaction can be qualified as benders or wrappers.[126] While in the case of wrappers (and notably for histone octamers within eukaryotes) the suggested electrostatic-induced DNA bending may be considered as the main mechanism involved, this is not always the case for benders. Indeed, DNA can be bent away from or around onto the bending protein according to the shape and charge distribution of the protein. Importantly, DNA wrapping (and bending to a lesser extent) is generally chiral (see below the nucleosome structure), hence generates some amount of supercoiling, which is constrained in the protein–DNA complex and does not require any active process (motor) for its maintenance. Note that proteins that can kink DNA have also an impact on supercoiling formation. Buckling transition occurs at significantly lower twist for kinked DNA molecules.[23]

A third class of architectural proteins is represented by DNA "bridgers", which are able to bind simultaneously to two dsDNAs and join them together. An example of such behavior is given in Fig. 5c for the bacterial protein Hfq. As described above for multivalent ions, electrostatics is the main actor of this bridging effect. And here again there exists a synergistic interplay between bridging and supercoiling. But, unlike multivalent cations, proteins have enriched features allowing a more complex behavior as *e.g.* a given degree of sequence specificity or the possibility of acting differently (as bender, wrapper or bridger) according to their monomeric or multimeric form. Moreover, the protein charge distribution may be tuned by post-translational modifications (as acetylation or phosphorylation), which in turn may finely tune its interaction with DNA.

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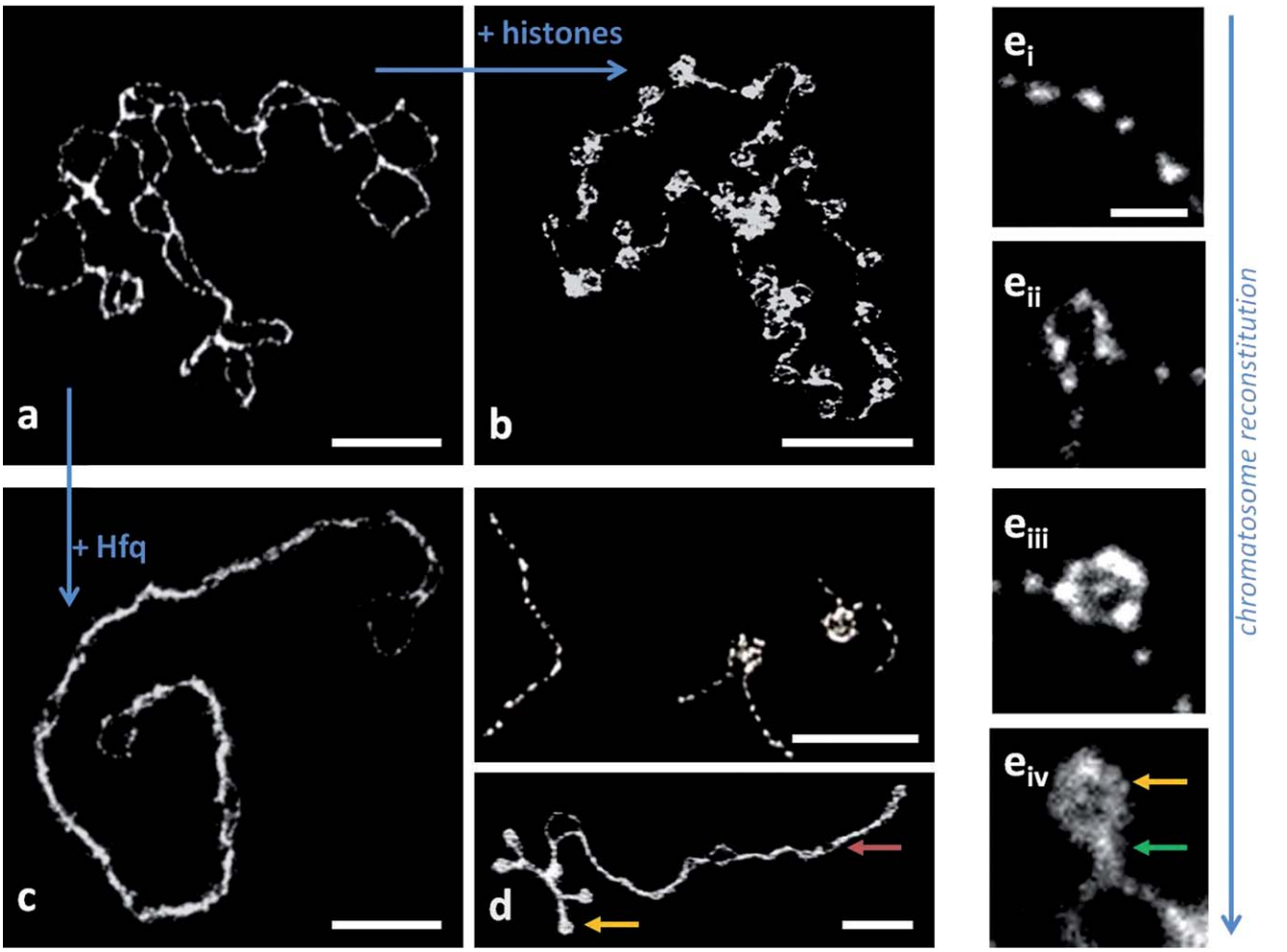

Fig. 5 (a) The folding of a naked plasmid in solution depends on its amount of supercoiling and on the ionic conditions. Here, a 5400 bp supercoiled plasmid was spread in bi-distilled water, showing a typical entangled conformation. (b) When core histones (purified from eukaryotic nuclei) are added, DNA wraps around them in typical round-shaped octameric particles (so-called nucleosomes) made from two of each histone type H2A, H2B, H3 and H4.[117] (c) The same plasmid incubated in the same condition with the "bridger" protein Hfq (purified from *E. coli* nucleoids) shows instead a typical bridged structure.[70] (d) Remarkably, some proteins from bacteria also wrap DNA in a "nucleosome-like" manner, as clearly seen from LrpC binding to short linear DNAs (upper part).[11] Indeed, LrpC was shown to form tetramers around which DNA wraps in a right-handed manner, causing an increase in the free negative supercoiling as seen from the tight winding of the free DNA part – red arrow – compared with free DNA in panel (a) to compensate for the LrpC-restrained positive supercoils – yellow arrow.[11] (e_i–e_{iv}) *In vitro* chromatosome reconstitution shows a three step process: (i and ii) a $(H3–H4)_2$ tetramer binds to DNA, (ii and iii) two H2A–H2B dimers join the structure, (iii and iv) eventually, a linker histone (H1) comes and seals the two linker DNAs. Note that core histones (H2A, H2B, H3, H4) together act as DNA wrappers (yellow arrow), while H1 acts as a bridger (green arrow). Scale bars: (a–d): 50 nm; ($e_{i–iv}$): 10 nm. Picture (d) by courtesy of Eric Le Cam.

3.3 A major architectural pattern: the nucleosome

Histone-like proteins in prokaryotes and archaea and histone octamers in eukaryotes carry large positive charges, which are responsible for strong electrostatic interactions with DNA. Many of these structural proteins bend DNA and work as DNA "benders" or "wrappers", depending on the degree of distortion induced on the DNA. In their electrostatic model, Arcesi and co-workers[4] considered the examples of an archaeal protein and of the histone octamer complex, and the result is rather close to the geometry actually observed. A schematic view of these geometries is shown in Fig. 3c and d, while Fig. 5e shows an example of nucleosome formation *in vitro*. This suggests a key role of electrostatics in tuning the specific geometry of DNA–protein complexes. However, this "non-structurally focused" view of the histone octamer simply bearing 146 net positive charges (many of which are on the tails) should not occult the fact that binding of DNA to the octamer is primarily mediated through a series of fourteen discrete binding sites (referred to as "minor groove-in positions"[82] or "superhelix axis locations"[124]) made by arginine residues spaced more or less evenly around the octamer. These residues form a left-handed superhelical ramp of a charged side chain that inserts between two phosphates into the minor groove of each DNA double helical turn.

The nucleosome reconstitution process *in vitro* can be seen as a complexation between an excess of polyanions (146 bp of DNA per nucleosome, with a net charge of $-294e$) and a limited pool of polycations (an octamer of basic histones per nucleosome,

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with a net charge of +146*e*).[110] So the nucleosomal complex is overcharged by the DNA.[205] A third ingredient is needed to allow the proper deposition of positively charged histones on to the negatively charged DNA and avoid non-nucleosomal aggregates that tend to form spontaneously when histone and DNA are simply mixed at low ionic strength. This "chaperone" function is usually mediated by histone-binding proteins (so-called "histone chaperones"), such as NAP-1 or nucleoplasmin,[221] negatively charged polymers (such as polyglutamate or RNA) or a high concentration of monovalent salts (such as NaCl). In the last case (known as salt gradient procedure), the core histones are initially combined with DNA in 2 M NaCl, then the NaCl concentration is slowly decreased by dialysis or dilution of the mixture.[183,214] Interestingly, this method recently inspired a new way to monitor the electrostatic complexation of anionic nano-particles by cationic–neutral block copolymers, which allows the formation of clusters with a size controlled by the desalting kinetics.[65]

3.4 Liquid-crystal ordering of DNA and nucleosomes

In vitro, pure DNA can form multiple liquid crystalline phases when the concentration is increased by osmotic stress. The two most typical phases are a left-handed cholesteric organization, with a pitch typically ranging from 2 to 3 microns,[122] and an hexagonal compact form (see Fig. 4a). The cholesteric pitch depends on both steric constraints due to the DNA right-handed helicity and electrostatic contributions. Isolated nucleosome core particles (NCPs) as well can form liquid crystalline phases under osmotic stress (*i.e.* by increasing the nucleosome concentration, for example by adding crowding agents such as PEG or dextran). They were shown to stack one upon each other, forming columns which then organize laterally to form an hexagonal packing[130] (see Fig. 4b). These phases can also be obtained by aggregation of the NCPs by multivalent cations.[13] In the case of chromatin, nucleosomes are regularly spaced along the genome and the contributions of both DNA and NCPs are expected to aid compaction. Indeed, a regular nucleosomal array is known to fold *in vitro* in a compact 30 nm chromatin fiber if divalent cations and the linker histone H1 are present.[188] All-atom modeling of the fiber[244] tends to argue in favor of the idea that the fiber stability results from an interplay between a short pitch cholesteric DNA liquid crystalline phase of linker DNA and a columnar phase of nucleosomes stacked in helices (see Fig. 4c). The pitch of the DNA helix can either be left or right handed depending on the linker length. Recent experiments on the formation of liquid crystalline phases of short DNA tend to support this view.[249]

4 *In vivo* implementation

4.1 Viruses

While the genome is highly confined spatially in all living organisms, the highest level of compaction is observed in the simplest possible organisms: viruses (see Table 1). During the assembly of many dsDNA viruses, the genome is compacted to near liquid crystalline density into a viral capsid (tens of thousands of base pairs for a total contour length of several microns packed into 50–80 nm capsids or virus heads).[54] Significant DNA bending must occur during DNA packaging; moreover, a repulsive electrostatic barrier, due to the negatively charged phosphate backbone, must be overcome.[105,162,177,178,185,233] This effect is partially reduced since the capsids of many dsDNA viruses are penetrable for small ions.[2] The presence of di- and trivalent cations in solution can therefore enhance the DNA–DNA electrostatic attraction and ease DNA packaging into the capsid. Neutralization of negative charge is generally done either with polyamines[7] and/or cations,[248] whereas some viruses partially neutralize the DNA charge with a positively charged domain of the capsid protein.[240]

As mentioned in Section 3.1, cations or polycations can spontaneously collapse DNA *in vitro* into folded-fiber (toroidal) forms provided the separation of a DNA phase occurs. Polylysine and the naturally occurring polyamine spermidine, in particular, have been reported to collapse DNA into a rod-shaped or toroidal structure.[73,84,113,163,246] On the other hand, electron microscopic studies have revealed a similar toroidal arrangement of the DNA inside certain bacteriophages and animal viruses.[37,67,106,119,184] This raises the possibility that spermidine and spermine are responsible for viral compaction *in vivo*,[56] since these polyamines are normally found in the host cell as well as in the mature virion.[2,72]

In spite of these effects, the size of the viral capsid is close to the persistence length of the DNA, and a strong energy barrier has to be overcome during viral assembly. The viral genome packaging is indeed achieved by an ATP-powered molecular motor that translocates dsDNA into the preformed viral capsid.[28,210] This process can be assimilated with the formation of a DNA liquid crystalline mesophase using an osmotic stress (see Fig. 4). A detailed investigation of the role of electrostatics in single DNA packaging was carried out in bacteriophage Φ29 *via* optical tweezer measurements,[66] showing that the internal force resisting DNA packaging was lowered when Mg^{2+} was the dominant ionic species or by addition of 1 mM Co^{3+}. This force was up to 80% higher with Na^{+} as the dominant counterion and only 90% of the genome length could be packaged.[66] This study revealed that ions also affect motor functioning; Mg^{2+} is required for initiation of packaging, while Na^{+} increases the motor velocity by up to 50%. Mg^{2+} is indeed known to be a cofactor for motor functioning as is the case with polymerase helicase and endonuclease.[39]

4.2 Prokaryotes

In prokaryotes, DNA is not enclosed by a membrane, but occupies an irregular region of the cell called the nucleoid. Prokaryotic genome is generally circular (possibly in multiple copies). It is negatively supercoiled, and shows distinct domains topologically independent, which have dynamic boundaries but an average size of 10 kbp.[230] Such a high level of negative supercoiling can be obtained thanks to the action of a specific bacterial enzyme, the DNA gyrase, that has the unique capability of introducing negative supercoils in DNA.[71] High valence ions such as spermines and spermidines contribute to lower the electrostatic DNA–DNA repulsion and thus facilitate supercoiling and compaction in bacteria; however, DNA compaction implies the action of nucleoid-associated proteins (NAPs) that, together with DNA, form the bacterial nucleoid.[21,48,125,213,230]

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There are about twelve NAPs involved in the formation of the nucleoid.[6] Some of these proteins are distributed uniformly within the nucleoid, while the others show an irregular distribution.[5,238] NAPs' contribution to the overall structure of the bacterial chromosome *in vivo* is unclear, since most details about how they interact with DNA come from *in vitro* studies.[43] Some NAPs constrain supercoils and some do not.[165,206,218,232] Most of them act as benders or bridgers, and therefore possibly facilitate and stabilize supercoiling (see Fig. 1).

The most abundant NAP is the small H-NS (histone-like nucleoid structuring protein), present in >20 000 copies per cell,[186] that is about one protein per 400 bp. H-NS is a DNA bridger that acts as a dimer[126] and compacts DNA.[49,186] Due to the presence of two DNA binding domains, the protein can interact with two DNA duplexes simultaneously.[44] The protein binds with relatively high affinity to any DNA sequence,[50] although some preferential binding sites have been reported.[19,114]

HU (heat-unstable nucleoid protein) and FIS (factor for inversion stimulation) are two DNA benders that act as dimers and can bend DNA up to 160° (ref. 126) and to 90°,[168] respectively. HU induces negative supercoils *in vitro* when incubated with DNA and topoisomerase I.[22] It exhibits no sequence specificity, but it has been shown to bind preferentially to intrinsically flexible DNA.[220] At high concentrations HU dimers can be packed tightly side-by-side in a cooperative manner resulting in effective stiffening of the DNA.[236] The HU/DNA co-crystal structure provides, at high resolution, an explanation for how HU may constrain negative supercoils, and evidences a proline intercalation and asymmetric charge neutralization mechanisms of DNA bending.[218]

When bacteria are grown under limited food conditions, they reach a phase called the stationary phase during which they stop growing and dividing. It was observed that, shortly after the onset of the stationary phase, bacterial chromatin undergoes a transition from a disperse morphology to several ring-shaped toroids that contain DNA and the protein Dps (DNA-binding protein from starved cells).[64] This condensation overall is driven in part by the loss of the transcriptional activity and by the natural tendency of DNA to condense at high concentrations in the presence of counterions. This type of condensation is similar to packaging in phage heads and bacterial spores, and probably constitutes one of the simplest ways of creating inactive bacterial chromatin.

Besides benders and bridgers, some DNA wrappers are also present in bacteria. Conserved examples are members of the Lrp/AsnC family. These proteins form disc-shaped octameric structures with multiple binding sites that wrap DNA around themselves in a right-handed superhelix.[46,223] An example of this behavior *in vitro* is given in Fig. 5d for protein LrpC. When two Lrp octamers associate, a hexadecamer is formed that can wrap DNA around itself by almost two full turns, resulting in a nucleosome-like structure.[223] Interestingly, members of the Lrp/AsnC family also appear to have a second mode of interaction with DNA that involves duplex bridging.[11,222]

4.3 Archaea

The archaeal and eukaryotic nucleosome core histones evolved from a common ancestor. The best studied example of an archaeal histone is the HMf protein. Two HMf polypeptides exist (HMfA and HMfB) that can form homodimers and heterodimers with different DNA binding properties.[197,198] Further assembly into tetramers allows wrapping of 60–70 bp of DNA,[77,174] in a similar way to eukaryotic tetrasomes $(H3 + H4)_2$ (ref. 1) (Fig. 3c). The wrapping (and consequent supercoiling) can be negative or positive, and depends on the histone-to-DNA ratio and salt concentration.[135,154] Interestingly, Soares and coworkers[211] substituted eukaryotic conserved lysine residues at their corresponding locations into the archaeal histone, HMfB, and obtained an increased affinity to DNA and a higher compaction level. At the same time, these complexes remained negatively supercoiled at all histone-to-DNA ratios, thus losing their flexibility to wrap DNA alternatively in either a negative or a positive supercoil. Both observations suggest a crucial role of these residues in the evolutionary differentiation of archaeal and eukaryotic histones.

Archaeal histones are considerably smaller than their eukaryotic counterparts and, with some exceptions, lack the C- and N-terminal tails. The absence of such tails suggests that archaeal histones are not subject to similar regulatory processes as found in eukaryotes. In line with this finding there is no evidence for post-translational modification of archaeal histone proteins.[199]

Besides HMf, other proteins participate in DNA packaging, such as MC1, an abundant histone-like protein co-responsible for the physiological structuring of the archaeal chromosome.[118] MC1 is a globular protein with a net positive charge of +12; its visualization by EM shows sharp kinks in DNA with a bending angle of 116° (or 0.32 turn).[34,91,169] Alba is another DNA condensing protein expressed in most archaeal genomes.[3] It forms regular fibrous structures with plasmid DNA, possibly wrapping two dsDNAs around each another.[127]

Interesting temperature-related features emerge in the case of hyperthermophilic archaea, living at temperatures of the order of 90 °C. If a high GC content is needed to resist to heat double-strand denaturation, intermolecular ion pairs formed between histidine and aspartate during histone tetramerization observed *in vitro* have also been suggested to play an important role in the maintenance of the archaeal nucleosome structure and for DNA stabilization.[87] Most interestingly, unusual long and branched polyamines are often found in hyperthermophile archaea. They are more abundant when cells are grown at higher temperature[164] and are thought to stabilize DNA against thermal denaturation.

4.4 Eukaryotes

In the case of eukaryotes, DNA wrapping is the essential compaction mechanism and it is achieved by specific octameric complexes made of histones. The different steps in the histone–DNA aggregation *in vivo* are illustrated in Fig. 5. The first organization level in eukaryotes is the nucleosome, which consists of a "nucleosome core particle" (NCP) of 146–147 base pairs of DNA wrapped around a roughly cylindrical core of eight histone proteins[45,124] and a linker DNA (stretch of free DNA between two NCPs, Fig. 3d and 5b). The NCP shows a tripartite structure: a $(H3–H4)_2$ tetramer binds to DNA (Fig. 5e_{ii}), then is complexed by two H2A–H2B dimers (Fig. 5b and e_{iii}). A linker histone, named H1, may eventually come and seal the two linker DNAs flanking a NCP (Fig. 5e_{iv}). Linker histone H1 and its

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variants[251] were shown to interact with chromatin transiently in the order of minutes.[120,146] The H1 globular domain contains two DNA binding patches on opposite sides of the protein, similar to the suggested structure of bacterial H-NS.[76,125] The presence of these two DNA-binding domains endows H1 with the ability to bridge DNA duplexes.[31]

Nucleosomes *in vivo* are rather regularly spaced along the genome, so that nucleosome core particles form a "beads on a string" array, also called "nucleosomal array".[62] The repeat length (*i.e.* the mean number of base pairs per nucleosome) may vary depending on organisms, tissues, and genomic regions, and is approximately in the range of 155 to 240 bp.

It is generally assumed that higher order structures of the nucleosomal array are involved in achieving an efficient and adjustable genome compaction. However these higher levels of organization remain largely speculative. Folding of the nucleosomal array into 30 nm chromatin fibers has been observed *in vitro*[103,189] and some indications of their existence *in vivo* have been recently obtained,[203,204] but the subject is still debated.[128] The issue of the higher-order structure of chromatin is directly related to the characterization of nucleosome–nucleosome physical interactions. The ability of nucleosomes to stack their faces together has been earlier observed using electron microscopy on isolated NCPs.[52] As discussed in Section 3.4, this was later confirmed and more precisely quantified by the study of NCP liquid crystals (see ref. 123 for a review). The relevance of this nucleosome–nucleosome interaction within chromatin fibers has been proved by the presence of an 11 nm meridional band in the X-ray diffraction pattern of oriented fibers.[241] All the models for compact fibers are therefore based on the assumption that nucleosomes are stacked in columns which are further twisted to form the 30 nm fiber (see *e.g.* Fig. 4c). In the emerging picture, these stacking interactions are essential for the fiber integrity and can be tuned *in vivo* using both variant histones and post-translation modifications (see ref. 231 for a review).

While packaging of the genome in eukaryotes is primarily due to the wrapping of DNA around nucleosomes, members of the high mobility group (HMG) family of proteins play a supplementary, possibly modulatory, role in chromatin organization.[24,225] HMGs[172,182] (including HMGA family,[182] HMGB family[225] and HMGN family[26]) are DNA benders. At a distance of 2 bp from the first kink, a second kink is induced due to partial intercalation of two additional residues.[153] This induces a bend into DNA of up to 80° for a single HMG box and up to 130° for tandem boxes. While HMG box proteins can promote DNA compaction by bending,[209] a picture is emerging in which these proteins function by favoring the partially unwrapped state of nucleosomes by binding at the entry/exit point, which possibly facilitates nucleosome remodeling.[229] Other non-histone proteins participating in chromatin polymorphism and dynamics are HP1, SMCs,[92] DEK,[239] and BAF.[137]

Finally, it is worth noting that mitochondria, constituent organelles of eukaryotes, have a compaction strategy similar to that in bacteria, and use HMG-like proteins.[29,61]

5 Functional insights: tuning the DNA compaction

We have seen so far that DNA compaction in cells is highly driven by electrostatic properties of the proteins and ions. We can therefore expect that the functional tuning of this compaction is also driven through electrostatics. In this section, we wish to address this specific issue in eukaryotes focusing on three different aspects. We will first review the tuning of the protein local charge through post-translational biochemical modifications of aminoacids focusing our attention on the nucleosome. We then turn to the extreme chromatin condensation observed during cell division, which involves post-translational modifications and also the action of molecular motors. Finally, we will discuss some results on the role of cation concentration variations in tuning the chromosome compaction during the cell cycle.

5.1 The role of biochemical modifications

Cell differentiation, implied *e.g.* in the formation of different tissues, needs heritable changes in gene expression. These changes can be caused by mechanisms other than genetic modification of the nucleotide sequence, and are referred to as *epigenetic* modifications. These changes are typically related to chromatin structure and compaction, which in turn alter the gene expression. DNA methylation and histone modification are typical examples of such modifications.

Histone acetylation and phosphorylation effectively reduce the positive charge of histones and this has the potential to disrupt electrostatic interactions between histones and DNA. This presumably leads to a less compact chromatin structure, thereby facilitating DNA access by protein machineries such as those involved in transcription. Notably, acetylation occurs on numerous histone tail lysines.[112] Moreover, acetylation of Lys9 and Lys56 is correlated with response to DNA damage.[226]

The X-ray structure of the nucleosome indicates that highly basic histone amino (N)-terminal tails can protrude from their own nucleosome and make contact with adjacent nucleosomes.[124] Modification of these tails affects inter-nucleosomal interaction and thus affects the overall chromatin structure. Acetylation of lysine or phosphorylation of serine or threonine is one way to reduce the charge. Models predict that acetylation of the histone core will destabilize the nucleosome.[60] This is in agreement with recent *in vivo* experimental evidence which suggests that acetylation of lysine 56 or 36 of histone H3 is necessary for efficient gene transcription.[148,245] Moreover, acetylation of lysine 56 also enables DNA replication and prevents epigenetic silencing[51,145] consistently with a looser state of the nucleosome acetylated at lysine 56 in the theoretical model.[60] Conversely, deacetylation of lysine 56 of histone H3 leads to stronger compaction and to a transcriptionally silent chromatin (heterochromatin).[245] As another experimental example, acetylation of all H4 histone tails at lysine 16, a prevalent epigenetic modification in eukaryotes, causes charge reduction and in turn inhibits the formation of compact 30 nm chromatin fibers and hampers the formation of cross-fiber interactions.[208]

Moreover, modifications regulate the recruitment of remodeling enzymes. Acetylated lysines are bound by bromodomains, which are often found in HATs (histone acetylase) and chromatin remodeling complexes.[151] For example Swi2/snf2 contains a bromodomain that targets it to acetylated histones. In turn, this recruits the Swi/snf remodeling complex which functions so as to open the chromatin.[83]

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5.2 Chromatin compaction in mitosis (so-called "chromatin condensation")

Chromatin condensation and subsequent de-condensation are essential for proper execution of cell division, or mitosis. During mitosis chromatin is highly compacted, whereas it is de-condensed right after chromosome segregation. Phosphorylation of histone H3 is a key process driving mitosis and the histone H3 tail is phosphorylated at four specific residues.[40,74] Interestingly, phosphorylation at three of those residues is found in transcriptionally active genes. Thus, H3 phosphorylation is believed to be involved in two structurally opposing processes, chromatin de-condensation observed during gene transcription in interphase and chromosome condensation during cell division.[175] In mammalian cells, phosphorylation of H3 starts in the late G2 interphase. Subsequently, the phosphorylation of H3 spreads along the chromosomes and is completed in prophase. It is still present in metaphase.[86] The temporal and spatial relationship between chromosome condensation and phosphorylation has been clearly observed.[75] In addition, acetylation of H3 also occurs on the same H3 tail.[69]

Replicated chromosomes assembled in linker histone H1 depleted crude extracts are thinner and 50% longer than control chromosomes.[136] Moreover, H1 is hyperphosphorylated by mitotic cyclins.[16] These studies suggest a role of H1 in the mitotic chromosome structure. Since the existence of chromatin fibers in mitotic chromosomes has been challenged recently, based on two independent electron microscopy studies,[57,68] this role seems different from its role in stabilizing the *in vitro* compaction of nucleosomes into thicker 30 nm fibers.[224]

The mechanism that drives chromatin condensation during mitosis is still debated.[57,68,150] Molecular motors such as condensins and topoisomerases play an essential role in this process. Condensed mitotic chromosomes have been shown to be organized in loops of DNA anchored to a proteinaceous chromosome axis.[55,173] The mechanism by which condensins generate and maintain condensation remains controversial. The enrichment of the chromosome axis with condensins suggests that condensin bridges could stabilize these loops by anchoring them to the axis. Recent reports suggest that condensins first bind to DNA through electrostatic interactions and then trap distant DNA segments to stabilize chromatin loops.[41] It has been also suggested that the introduction of positive supercoiling by condensins could be the basis for mitotic chromosome architecture.[104]

5.3 Cations in chromosome condensation

Cations are essential participants in chromosome condensation.[139,140] Mitotic chromosomes become very swollen following the depletion of Ca^{2+} or Mg^{2+}. This process is completely reversible. It has been demonstrated that the repeated removal and addition of Mg^{2+} resulted in cycles of chromosome swelling and compaction.[35]

A report of the distribution of cations in nuclei and isolated mitotic chromosomes using secondary ion mass spectrometry[216] concluded that during the transition from interphase to mitosis, Ca^{2+} and Mg^{2+} concentrations increase 3 to 4 fold, reaching 18 mM and 11 mM respectively. Interestingly, mitotic chromosomes show axial enrichment in Ca^{2+}. As far as monovalent ions are concerned, Na^{+} concentrations are close to those measured in the cytosol, whereas K^{+} concentrations are clearly higher in both interphasic and mitotic chromosomes. It is also well established that polyamines especially spermidine (3+) and spermine (4+)[138,141] are ubiquitous components with potent chromatin compaction properties.

6 Evolutionary perspectives

6.1 One problem, many solutions

Viruses, bacteria (eu- and archaebacteria) and eukaryotes have all found ways to compact their genome, accumulating a spectacular amount of highly charged DNA into tiny volumes (see Table 1). Advantages of accumulating such a quantity of DNA with regard to the physical cost of compaction are not obvious, especially in organisms in which most of the DNA has not yet revealed its functional role. Hence, while bacteria genomes are very dense in transcribed sequences (intergenic regions are very small and there are no introns), this is definitely not the case for human genomes: one million base pairs of bacterial DNA contain 500 to 1000 genes whereas one million of human DNA contain only on average 10 genes. What may be the advantages of the presence of these intergenic regions for chromatin organization is still a debated question. It is noteworthy that many proteins bind to DNA through a shape-dependent recognition that depends on minor groove width and electrostatics. Hence, the molecular nature of DNA embeds a supplementary layer of information (distinct from the genetic information) that could dictate various important features such as nucleosome positioning and transcription factor binding. As such, DNA is subject to evolutionary constraint, even in non-coding regions.[170] As already discussed previously, various ways to compact DNA co-evolved in the different kingdoms, and prokaryotic and eukaryotic organisms may indeed not be as different as is generally believed with regard to their chromatin structure and properties.[12] For instance, the eukaryotic dinoflagellate *Crypthecodinium cohnii* that lacks histone proteins encodes HCC proteins (histone-like proteins of *Crypthecodinium cohnii*) that share homology with bacterial HU proteins.[243] Also, many bacteria contain homologues of the eukaryotic H1, such as Hc1 and Hc2 proteins found in Chlamydia,[80] whose nucleoid compaction capacity was further demonstrated.[9] *Bordetella pertussis* encodes two H1-like proteins (Bph1 and Bph2) and a H-NS-like protein (Bph3); *Pseudomonas aeruginosa* encodes the H1-like AlgP protein and five different H-NS-like proteins; *etc.* The reader interested in these comparisons between the various DNA benders/wrappers/bridgers found in different organisms should refer to the quite exhaustive review by Dame and colleagues.[126] From a phylogenetic point of view, the origin of histone H1 can be traced to bacteria whereas core histones are believed to originate from archaea.[98,199] H3 and H4 evolved at a far slower pace than H2A and H2B, reflecting their crucial role in the first steps of nucleosome formation.[129] Remarkably, further specializations of histone variants (mainly through differences in their DNA wrapping properties and specialized domains that regulate access to DNA) have evolved in some lineages to perform additional tasks, underlying the diverse functions that histones have acquired in evolution.[219]

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6.2 The invention of the nucleus and epigenetics

The nucleus is a highly organized compartment containing several sub-compartments thought to offer various specific micro-environments that regulate chromatin state and transcriptional regulation.[144,181] How does this arise? The presence of histones and nucleosomal packaging of chromosomal DNA probably predates the development of a nuclear membrane.[12] Then, according to the coevolutionary theory of eukaryote origins, the fundamental innovations were the concerted origins of the endomembrane system and cytoskeleton, subsequently recruited to form the cell nucleus and coevolving mitotic apparatus.[27,176,242] Historically viewed as little more than a diffusion barrier between the cytoplasm and the nucleoplasm, the nuclear envelope is now known to have roles in the cell cycle, cytoskeletal stability, genome architecture, epigenetics, regulation of transcription, splicing and DNA replication.[252] From an electrostatic point of view, much is still unknown of the interplay between lipid binding, counterion displacement, and DNA condensation.[142] DNA methylation appears as a strong epigenetic regulator in all kingdoms of life, including bacteria.[25] The enzyme responsible for methylation of histones H3 and H4, the histone lysine methyltransferase (KMT), utilizes a catalytically active site called the SET domain. SET domains have been shown to be involved in eukaryotic chromatin modification by methylating some histone lysine residues (Kouzarides 2002 (ref. 111)). The identification of SET domain methyl-transferase proteins in bacteria raises the possibility that epigenetic inheritance in these organisms may also involve DNA-associated proteins.[152] The demonstration of a physical interaction between the chlamydial SET domain protein and histone-like proteins Hc1 and Hc2 (described in Section 6.1) provides some evidence of a similar regulatory mechanism in bacteria.

At the same time, proteomic approaches have unraveled many unknown acetylation sites in bacteria and archaea.[212] As a whole, it seems that common mechanisms in gene silencing (the programmed inhibition of the gene transcription) are shared by all life forms,[247] although the logic behind these mechanisms has been argued to be quite different.[217]

6.3 Concluding remarks

Far from having "explained" the role of electrostatics in the making and tuning of functionally active levels of compaction in living organisms, we have tried to review the various ways by which electrostatics intervenes in these key biological mechanisms. Coming back to Fig. 1, we can now briefly sum up the interplay between the different actors evoked throughout the paper. The mechanical properties of DNA and the DNA–DNA interaction are both strongly determined by its high line charge density, and are therefore directly tuned by the ion screening effect. These properties in turn control DNA bending and twisting flexibility, and therefore the formation of supercoiled structures like plectonemes or toroids. In Section 4.2 it has been stressed that the active (motor dependent) maintenance of unconstrained supercoiling is a crucial compacting mechanism in bacteria.

Highly charged multivalent ions which induce DNA–DNA attraction strongly facilitate compaction by allowing the formation of bridges and liquid crystal ordering of dsDNAs. Moreover, these multivalent ions can *locally* modify the DNA structure, and in particular they can induce DNA bending. In this respect, they prefigure the second family of compacting agents, namely proteins. Indeed DNA binding proteins are multivalent ions that can bend DNA and moreover act as wrappers or bridgers. Unlike simple ions, DNA binding proteins have a new important feature: their specificity. Their "design" has been selected so as to bind DNA in specific sites, and – probably most important for the purpose of compaction – some of them contain specialized domains that can ensure dimerization, hence bridging of two dsDNAs specifically. Moreover DNA binding proteins can be bound to other proteins to form super-structures of increasing complexity. In eukaryotes, the nucleosome complex is the key component involved in the mechanism of compaction: the unconstrained supercoiling relevant to bacteria is replaced by constrained supercoiling, locally managed by protein–DNA interaction. Eukaryotic chromatin is a beautiful example of this "high-tech engineering": an inhomogeneous, multiscale, tunable architecture, made of protein–DNA and DNA–DNA electrostatic interactions and tuned by small ions and post-translational electrostatic modifications.

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