DNA packaging by molecular motors: from bacteriophage to human chromosomes

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Abstract | Dense packaging of genomic DNA is crucial for organismal survival, as DNA length always far exceeds the dimensions of the cells that contain it. Organisms therefore employ sophisticated machineries to package their genomes. These systems range across kingdoms from a single ultra-powerful rotary motor that spools the DNA into a bacteriophage head, to hundreds of thousands of relatively weak molecular motors that coordinate the compaction of mitotic chromosomes in eukaryotic cells. Recent technological advances, such as DNA proximity-based sequencing approaches, polymer modelling and in vitro reconstitution of DNA loop extrusion, have shed light on the biological mechanisms driving DNA organization in different systems. Here, we discuss DNA packaging in bacteriophage, bacteria, and eukaryotic cells, which despite their extreme variation in size, structure and genomic content, all rely on the action of molecular motors to package their genomes.

[H1] Introduction

All organisms rely on the precise packaging and 3D organization of their genome for survival and proliferation. Although they all share the problem that their DNA length far exceeds the diameter of the compartment that contains it, interestingly, packaging strategies and machineries differ widely. In bacteriophage lambda, ~50 kb of double-stranded DNA (dsDNA) is **lengthwise compacted [G]** about 250-fold, being spooled by a single motor until it reaches almost crystalline density inside the phage head[1]. In organisms ranging from bacteria to human, the much longer DNA is compacted 1,000–

10,000-fold to fit inside the nucleoid or cell nucleus by the coordinated action of many independent motor complexes, often involving structural maintenance of chromosomes (SMC) proteins. In vertebrates, a high level of **DNA compaction [G]** is already present in interphase cell nuclei (~3,000-fold lengthwise compacted), in part via the action of the SMC complex cohesin. However, during mitotic chromosome formation, the DNA is dramatically reorganized and compacted an additional 3-fold in a process referred to as **chromosome condensation [G].** This reorganisation relies on the coordinated activities of condensins, topoisomerase IIα and the chromokinesin KIF4A. Although the difference in DNA density between interphase and mitosis is relatively minor, the structural and functional reorganization of the genome during mitotic chromosome formation is profound.

Over the past decade, advances in biological and biochemical techniques coupled with advances in high-resolution microscopy and polymer modeling have dramatically improved our understanding of DNA organization and the structure and functioning of DNA-interacting proteins in different systems. For example, recent high-resolution cryo-electron microscopy (EM) structures have revealed the molecular structure of the bacteriophage rotary motor[2–4], and highly sensitive single-molecule techniques such as optical tweezer assays have defined its mechanical parameters[5–7]. Our understanding of DNA compaction in bacteria has been propelled by single-molecule fluorescence studies (in vitro and in vivo), which have allowed the quantification of the numbers and properties of the proteins involved, including the dynamics of individual SMC complexes[8,9]. Furthermore, next-generation sequencing and Hi-C have enabled us to determine where and when those complexes act and how the bacterial chromosome is organized in vivo[10–12]. In eukaryotic cells, techniques including Hi-C, high-resolution fluorescence microscopy and polymer modeling have provided detailed structural insights into mitotic chromosome formation and have begun to reveal the underlying mechanisms, which involve DNA enzymes and molecular motors[13–16]. Moreover, an emerging field of single-molecule studies has documented the ability of purified SMC complexes, acting alone or in combination, to compact the DNA by forming loops in vitro[17,18]. Together, these recent high-resolution, quantitative studies have been used to visualize the dynamics of DNA

packaging and organization across a huge range of genome sizes. However, there remain gaps in our understanding of how the collective behavior of DNA motors results in the overall structural (re)organization of chromosomes.

In this Review, we briefly introduce and contrast key discoveries and concepts concerning DNA organization across kingdoms, namely in bacteriophages, bacteria and eukaryotic cells. We set out some of the major problems that each system faces in order to package its DNA, introduce key motor components involved, and describe some of the exciting new studies that reveal how these motors achieve the desired DNA compaction. We introduce several key quantitative methods that have been employed and summarize essential insights derived from them. We hope that the examples presented here will leave readers with an improved mechanistic understanding of the diverse solutions to the problem of how motors compact chromosomes.

[H1] The challenge of packaging DNA

Chromosomes are unineme, that is, they consist of a single uninterrupted DNA double helix. This was first demonstrated by Gall in 1963, who quantified the kinetics of DNA fragmentation of lampbrush chromosomes during DNA digestion[19]. It should be noted that he designed a custom-built inverted microscope for those studies, one of the first examples of changes in microscope technology that enabled novel insights into chromosome structure. It is now known that DNA double helixes are very densely packed, resulting in about 1,000–10,000-fold lengthwise compaction of the chromosome, relative to its **contour length [G]**[13].

Part of the challenge of achieving this level of DNA compaction becomes clear when we consider the physical properties of double stranded (ds) DNA. The DNA double helix consists of two intertwined sugar-phosphate backbones that pair via interstrand hydrogen-bonding between DNA bases projecting from each backbone. Local rigidity of the dsDNA arises from the stiffness of the sugar-phosphate backbone itself as well as intra-strand stacking interactions between the aromatic rings of adjacent DNA bases.

Taken together, this gives the 2 nm dsDNA filament a persistence length of about 50 nm (~150 bp) under physiological conditions (note that persistence length depends on ionic strength)[20]. Formally, this means that vectors tangent to the DNA within this length are correlated[21] and DNA behaves as a structurally rigid object within this length scale. Beyond this length, thermal fluctuations dominate and the correlation between tangent vectors is lost.

If we look at the definition of the persistence length:

$$\xi_{\mathrm{p}} = \frac{\kappa}{k_B T} \qquad (1)$$

where κ is the bending stiffness and $k_{\mathrm{B}}T$ the molecular thermal energy (~4.1 pN nm), it follows that DNA bending at length scales shorter than its persistence length requires the input of additional energy in the form of work (that is, an external force must be applied to induce further bending). Interestingly, this so called 'tight bending' of DNA is a common phenomenon in biological systems[22]. For example, in eukaryotes, each nucleosome wraps ~150 bp of DNA (the persistence length of DNA) almost twice around its 10 nm core[14,23], whereas in bacteriophage lambda ~50 kb of DNA is packaged into a viral capsid only ~50 nm across[1] (**FIG. 1a**). Unexpectedly, recent in vitro experiments have now shown that efficient DNA loop formation can occur in DNA filaments of <100 bp. This might reflect local changes in DNA conformation such as transient single-bp mismatches or kinks not adequately captured by the theoretical framework[24]. Moreover, estimates of the persistence length of chromatin — a complex polymer containing both negatively charged DNA and positively charged histone proteins — vary widely, between 30 to 220 nm[25–27]. This complicates our understanding of the forces involved in DNA bending in vivo.

Besides the energy required for tight bending of the DNA, packaging of DNA at near crystalline densities (as occurs in bacteriophages, see section below) comes with two additional energy costs[28]. First, DNA is a highly negatively charged polymer, with each base pair carrying two negative charges. Inside the cell, this negative charge is normally screened due to the presence of counterions (such as K^+, Mg^{2+} and polyamines)

with the spacing between adjacent DNA helixes normally exceeding the Debye length. The Debye length of DNA is the length within which an adjacent molecule would 'feel' the presence of the DNA, in other words the range within which electrostatic interactions can occur (that is, charge-charge repulsion for DNA-DNA interactions)[28]. This length is estimated to be ~2 nm depending on the chemical properties of the medium (cytoplasm or nucleoplasm)[28,29]. Therefore, the tight bending of DNA around nucleosomes is helped by the net positive charge of the histones, although other interactions such as hydrogen bonding may also play a major role[30].

In general, the structural properties of the DNA, in combination with the DNA volume density and required maintenance of DNA accessibility for transcription and replication, place powerful constraints on the organization of DNA and its associated components. It should be noted, however, that the structural properties of naked DNA and the associated theoretical framework might not directly translate to the situation in vivo. We begin by discussing DNA packaging by bacteriophages to highlight the implications that arise from super-tight packaging of functionally inert DNA into very small compartments without the assistance of DNA-associated proteins.

[H1] DNA organization in bacteriophages: a single DNA packaging motor

Many bacteriophages package their genetic material as dsDNA inside compact icosahedral heads (**FIG. 1a**). Other bacteriophages (not discussed here) have single-stranded DNA (ssDNA) or RNA genomes. During the infection cycle, DNA is released into the infected cell, where it is transcribed and replicated. Initially, an empty viral capsid is assembled, and subsequently the DNA is loaded and packaged into it by a single powerful molecular motor[5,31,32]. Gentle lysis of bacteriophage heads results in the DNA bursting out and spreading over a large area, indicating that the DNA inside must be highly compacted[33] (**FIG. 1b**). The bacteriophage system is of special interest, as the DNA inside the viral capsid is highly organized but this solely relies on the properties of DNA as it is constrained by the capsid. Thus, phage DNA organization does not involve

any DNA-binding proteins (other than the packaging motor). This is in sharp contrast to DNA packaging in bacteria and eukaryotes (discussed below).

Early studies using X-ray diffraction on bacteriophage T2 revealed that the DNA inside the viral capsid is tightly packaged and possibly present in a highly organized form[34]. Small angle X-ray scattering of P22 phage heads confirmed that viral DNA is highly organized with a long-range packing periodicity correlating with the internal diameter of the head[35]. Later work, using modelling of the X-ray diffraction patterns shed further light on the local packing geometry of P22 DNA[1]. By fitting a **fourier shell model [G]** to the diffraction data, the authors revealed that the ~2.5 nm short-range packing periodicity could be best explained by a local hexagonal arrangement of the DNA helixes (**FIG. 1c**). This arrangement allows the DNA in the head of P22 and other bacteriophages[36,37] to become so tightly packed that it resembles the hexagonal arrangement of DNA when in its highly concentrated liquid-crystalline phase[38]. Several models of DNA organization were proposed and it was concluded that this level of DNA organization was most likely achieved through inverse spooling of the DNA perpendicular to the longitudinal axis of the phage. In its most simplistic view, inverse spooling involves packaging of DNA in concentric circles, starting from the outside to the inside with each additional layer of DNA maximizing its radius and shifting laterally by ~2.5 nm until the head is full (**FIG. 1d**). In reality, the DNA packaging process occurs in slightly more chaotic fashion and final long-range DNA packing order is only established once the head is 70-100% full[39]. **Molecular dynamics [G]** simulations addressing how DNA rotation during loading affects final DNA packing order, have now provided striking visual insights into how this process might occur[40]. Moreover, the DNA organization (for example the orientation and precise conformation of the 'DNA spool') is highly dependent on the capsid dimensions and other packaging arrangements, similar to toroidal DNA packaging in T4[41] (**FIG. 1a, inset**).

The extremely high DNA packaging density in phage heads, with DNA helixes being only 0.85 nm apart, leads to significant electrostatic repulsion, which greatly increases the amount of energy required to pack the DNA[28]. In fact, electrostatic repulsion

is the main energy barrier that must be overcome during DNA packaging: the amount of energy required for DNA bending is more than an order of magnitude smaller[42]. Furthermore, upon completion of DNA packaging, the DNA occupies almost the entire capsid volume, severely limiting the number of available configurations it can adopt. This results in an additional entropic penalty[28,43]. As described below, in eukaryotes the electrostatic repulsion problem is partly dealt with by complexing the DNA with positively charged histone proteins.

Altogether, the high electrostatic repulsion in combination with the high DNA density leads to internal capsid pressures of several MPa[37,44], roughly similar to the pressure one would feel when an adult-sized African elephant steps on your toes. Therefore, extremely powerful molecular motors are required for the packaging of the DNA, especially in the later stages when internal capsid pressures are at their highest[6,37]. For excellent reviews on DNA packaging in bacteriophages see[2–6,31,32,36,37,45,46].

[H2] Packaging of DNA by the bacteriophage portal motor

During bacteriophage assembly, a single DNA portal is positioned on the outside of a 5-fold capsid vertex. This forms the 'DNA translocating vertex' or 'portal vertex' required for DNA translocation[4,47]. The portal ring, which exhibits dodecameric (12-fold) symmetry and makes up the proximal part of the motor complex, contains a narrow central channel to allow for translocation of the DNA[4] (**FIG. 1e**). This ring provides the foundation for viral head assembly. Interestingly, the portal ring is dynamic and can change its internal diameter to adopt an 'open' or 'closed' state, although the underlying mechanism is unclear[4]. Over the past several decades, EM studies have revealed intricate structural details about both the assembly and the complete structure of the capsid as well as that of the portal protein[2–5,32,45,46], however, these are beyond the scope of this review. After capsid assembly, 5 large and 8 small terminase subunits dock to the portal protein to complete the full DNA packaging complex[46] (**FIG. 1f**). The large homopentameric ring contains the motor subunits (ATPases I-V), belonging to the ASCE (additional strand

conserved glutamate) ATPase superfamily[3,5]. They interact with the DNA and provide the power to drive DNA translocation[3,5,45,46,48,49]. The smaller homo-octameric terminase ring, located distal to the ATPases, is required for initial substrate recognition and DNA binding[45,46,49].

Development of a highly sensitive single-molecule assay was key to obtaining detailed quantitative information about the bacteriophage motor during DNA packaging. In a hallmark study, Smith and colleagues tethered a ϕ29 bacteriophage between two microspheres to measure the forces involved in DNA packaging[50]. One of the microspheres was connected to the bacteriophage head using antibodies and held by a static micropipette. The other microsphere was connected to the end of the unpackaged DNA (using streptavidin-biotin) and held by an optical trap (**BOX 1**). Upon addition of ATP, the DNA packaging motor started to reel in the DNA-tethered microsphere, thereby exerting a force pulling the microsphere away from the center of the trap. Using this assay, the authors monitored real-time performance of the ϕ29 bacteriophage motor while it was operating under continuously increasing load due to the building up of internal capsid pressure. Parameters such as its maximum packaging rate (~100 bp/s) and average **stall force [G]** (~57 pN) could be accurately determined. In more recent studies using improved optical-tweezer assays, an even higher maximum packaging rate (~165 bp/s) (**FIG. 2a**) and stall force (~110 pN) (**FIG. 2b**) were measured for the ϕ29 DNA packaging motor[51]. To put this stall force into perspective, other DNA-interacting motor proteins such as condensin and cohesin (discussed later in this review) already stall at around 1 pN (**FIG. 2c**). As a caveat, it should be noted that all of these parameters measured in vitro are highly dependent on the ionic conditions of the reaction milieu[52].

Optical tweezer-based assays have subsequently been used in several other studies to further unravel the molecular mechanisms of bacteriophage motors. One study showed that bacteriophage T4 (**FIG. 1a**) has a stall force similar to bacteriophage lambda (**FIG. 2c**), however, the maximum packaging rate of T4 was found to be much higher compared to other phages, approaching 2 kbp/s[53] (**FIG. 2c**). As T4 has a relatively large genome (168.9 kb[54]) compared to ϕ29 (19.3 kb[55]), this suggested a correlation between

genome length and the DNA packaging rate, which could be crucial for the timely packaging of the DNA during the infection cycle[53]. Furthermore, pausing and slipping events were observed, as well as changes in the velocity of DNA translocation, leading to the hypothesis that these might allow for the completion of other DNA-related processes (or reorganization of the DNA within the head) while the strand is being packaged.

How the bacteriophage motor couples ATP-hydrolysis and associated motor subunit conformational changes to translocation of the dsDNA is an important question. The mechanochemical cycle of the ϕ29 DNA packaging motor was carefully dissected by measuring its activity under various externally applied loads using a range of different concentrations of ATP and AMP-PNP (a nonhydrolyzable ATP analog)[56–58]. These experiments revealed that the activity of the five ring motor subunits is tightly coordinated, with one subunit having an ATP-dependent regulatory role during the **dwell phase [G]**, and the remaining four driving a 10-bp translocation of the DNA (approximately one helical turn) in four discrete 2.5-bp steps during the **burst phase [G]**, powered by the hydrolysis of four ATP molecules. Further dissection of the DNA translocation revealed that the motor subunits maintain contact with adjacent phosphates on only one of the DNA strands during the dwell phase, contributing to the regulation of the mechanochemical cycle[59]. To assist proper packaging, the ϕ29 motor was found to rotate the DNA during translocation and to downregulate its ATP-binding rate to adjust or 'throttle-down' at final stages of DNA packaging[60]. A recent review drew from the aforementioned work as well as more recently published data to propose 'helical inchworming' as a translocation mechanism for a bacteriophage DNA packaging motor[7]. In this model, the subunits of the DNA packaging motor cycle between an extended helical conformation (matching the helical structure of the DNA) and a flat closed-ring conformation (after one full helical turn of DNA has been passed) in order to maintain their grip throughout the motor's mechanochemical cycle.

In summary, DNA packaging by bacteriophages occurs in the absence of DNA-interacting proteins other than the motor complex, which achieves a final packing density

mainly determined by the length of the genome and the size of the capsid. The high DNA packing density is thought to contribute to DNA ejection during infection by forcefully driving initial entry of the DNA into the host, however, the precise ejection mechanism and the independent force contributions involved remain under investigation[37]. In the next section, we describe DNA organization in bacteria, which use DNA-interacting proteins to maintain internal genome order.

[H1] DNA organization in bacteria: multiple motors producing a dynamic chromosome

The DNA of most bacteria is a single circular unineme molecule (0.1 to 15 Mb in size), which is condensed >1000-fold to form the bacterial **nucleoid [G]**[61,62]. However, in contrast to the bacteriophage genome, the nucleoid DNA must remain constantly accessible to allow for DNA-related transactions, including transcription and replication, to occur. Therefore, a more dynamic and less constrained form of DNA organization is required. This is achieved by the activity of various nucleoid-associated proteins[10]. These proteins allow the bacterial nucleoid to adopt a specific domain-like structure that is crucial for faithful distribution of the bacterial genome during cell division[62,63]. In this section, we discuss key DNA organizational features of three different bacterial systems: *Escherichia coli*, *Caulobactor crescentus* and *Bacillus subtilis*.

[H2] Fluorescence microscopy reveals E. coli chromosome dynamics

The first visual insights into bacterial genome organization came from early EM studies that imaged the DNA released when *E. coli* cells were lysed in situ[64–66]. These experiments revealed strikingly complex DNA structures, which extended far beyond the boundaries of the cell ghost, reflecting the extent to which the intracellular DNA inside was compacted as well as the major entropic penalty associated with that compaction (**FIG. 3a**). In these EM images the unfurled DNA consisted of a series of supercoiled loops

emanating from a central scaffold, although how DNA was organized inside the living bacterium remained unknown.

Twenty-five years later, fluorescence microscopy analysis of the 5 Mb circular *E. coli* genome provided detailed insights into this question. Researchers used fluorescence in situ hybridization (**FISH [G]**) to visualize 22 different DNA segments of the *E. coli* genome[67]. They discovered that the chromosome forms a compact ring-like structure having two distinct **macrodomains [G]** corresponding to the Origin (Ori) and Terminus (Ter) of DNA replication, which displayed cell-cycle dependent positioning. A later genetic study, identified the same Ori and Ter macrodomains plus two additional macrodomains, called the Left and Right macrodomains, as well as two non-structured regions[68]. To better understand the positioning of all these domains, fluorescent tags were inserted at various genomic locations, enabling live tracking of these loci (including those belonging to the specific macrodomains)[69]. In this way, it was discovered that macrodomains are much less dynamic compared to non-structured regions, and that upon cell division the macrodomains gradually move towards specific cellular locations, whereas the non-structured regions move more irregularly.

In *E. coli*, the four macrodomains contain a few hundred topologically isolated domains (each ~10 kb on average)[70]. The centrally positioned Ori macrodomain is linked to the Left and Right macrodomains via two non-structured regions, and the Ter macrodomain connects the Left and Right macrodomains to circularize the chromosome[62,67–69] (**FIG. 3b**). In slow-growing *E. coli*, the Ori and Ter macrodomain are typically centrally positioned with the Left and Right macrodomains on either side[11,62,63]. Interestingly, the macrodomains not only display cell-cycle dependent positioning but also have differences in their individual protein-dependent DNA organization and dynamics. For example, compaction of the ~800 kb Ter macrodomain is driven by the specific nucleoid-associated protein, MatP, which bridges its 23 scattered 13-bp motifs, known as *matS* sites[71,72] (**FIG. 3b**). It should be noted, however, that the two other bacterial systems discussed below feature a slightly different macrodomain organization, and organize their genomes mainly using a parB/*parS*-based system, which is absent from *E. coli*[11].

[H2] DNA organization and DNA supercoiling in C. crescentus

In *C. crescentus*, the Ori and Ter macrodomains of the 4 Mb circular genome are positioned at either end of the rod-shaped bacterium, with the Left and Right macrodomains running along the long axis[11,62,63] (**FIG. 3b**). One study used fluorescence live-cell imaging to monitor the position of 112 individual LacI-GFP-tagged loci distributed across the chromosome over the course of a cell-cycle[73]. The authors found that each locus occupies a preferred cellular location correlated with its genomic position. A decade later, researchers provided insight into the 3D genome organization of *C. crescentus*, using a combination of chromosome conformation capture (3C) technology (**BOX 2**), polymer modeling and fluorescence microscopy[74]. They confirmed that *C. crescentus* genomic loci do have preferred longitudinal locations but lack preferred radial locations.

A landmark study increased the spatial resolution even further by using Hi-C (**BOX 2**) and polymer modeling to greatly refine our understanding of the 3D spatial organization of the *C. crescentus* genome[75]. It was found that Hi-C data were best explained if the DNA was arranged into a fiber consisting of a series of ~300 spatially isolated supercoiled **DNA plectonemes [G]** (**FIG. 3b**), each about ~15 kb in length, interspersed by small <300 bp plectoneme-free regions. The supercoil-free regions were enriched in highly expressed genes, thereby correlating gene expression to the 3D structure of the bacterial DNA. As plectonemes are spatially isolated structural domains, they prevent entanglement of the DNA and are therefore a key organizational feature of genome organisation. Surprisingly, the plectonemic DNA supercoils in the bacterial genome are very dynamic, with RNA transcription functioning as a barrier to block their diffusion[76,77]. Thus, plectonemes are often found at transcription start sites and recent studies have now also shown that their positioning is DNA-sequence dependent in vitro[78]. Moreover, it was found that plectonemes are also dynamic in vitro, diffusing along the DNA or suddenly disappearing and nucleating at an alternate position, with plectoneme number and dynamics being DNA-tension dependent[79].

316 Together, these observations give a view of the bacterial chromosome as a
317 dynamic, constantly changing entity rather than a single static structure.

318 ***[H2] DNA supercoiling and topoisomerases***

319 **DNA supercoiling [G]** is an important factor in genome organization that affects DNA
320 accessibility and important processes such as gene expression and genome replication[80–
321 84]. Supercoiling arises from the double helical properties of the DNA and the activities of
322 DNA-associated machinery manipulating it[81,83]. In solution, a small piece of dsDNA with
323 both ends free, will adapt a so called 'relaxed state', with one helical turn every ~10.5 bp,
324 reflecting its intrinsic (sequence-dependent) structural properties. Because the two
325 strands of dsDNA are wound around one another in a helix, processes such as
326 transcription, which involve peeling apart the two strands, exert a torque that can lead to
327 the local overwinding (positive supercoiling) or underwinding (negative supercoiling) of
328 the DNA, and subsequent formation of plectonemes, if not resolved[76,85–87]. For every
329 ~10.5 bp transcribed, one positive DNA supercoil is generated upstream and one
330 negative supercoil downstream of the polymerase. Indeed, transcription alone can
331 introduce up to ~3,700 DNA supercoils per second into the *E. coli* genome (assuming a
332 ~45 bp/s transcription rate[88] and ~430 active RNAPs[89]). As DNA supercoiling influences
333 many DNA transactions, supercoiling levels are tightly regulated[81–83].

334 DNA topoisomerases are enzymes that alter the topological state of the DNA and
335 can introduce or relieve DNA supercoiling or entanglement[90–92]. They are divided into two
336 types: type 1 topoisomerases, which act on single DNA strands, and type 2
337 topoisomerases, which create and ligate double-stranded breaks in the DNA in an ATP-
338 dependent manner, allowing for strand passage to occur[91,92]. DNA gyrase, a type 2
339 topoisomerase that is found exclusively in bacteria, can directly induce negative
340 supercoiling in relaxed closed-circular DNA[93]. DNA gyrase preferentially targets positive
341 DNA supercoils and can directly convert them into negative DNA supercoils[90]. Single-
342 molecule fluorescence microscopy experiments reveal that ~600 gyrase enzymes are

present in *E. coli.* About half were found to be tightly bound to the genome, with about ~12 gyrases enriched near each replication fork to control DNA supercoiling (each fork generates up to 100 (+) and 100 (-) supercoils per second)[94]. In addition to their involvement in controlling DNA supercoiling levels, topoisomerases are important to resolve DNA links, knots and entanglements that could be detrimental to the cell. Type 2 DNA topoisomerases also have key roles in eukaryotic chromosome organisation.

[H2] SMC complexes and DNA organization in E. coli and B. subtilis

In 1990, Riggs[95] proposed that enzyme-based DNA translocation ("DNA reeling") could provide specificity to chromosome folding by arranging the DNA as a series of looped domains. A few years later, Guacci et al. hypothesized that SMC complexes might be the motor proteins responsible for driving DNA loop formation[96]. This inspired a 'coiling model for SMC function' by Peterson[97]. However, it was Nasmyth who first proposed the explicit idea that SMC motor activity might act at the core of chromosome organization by processively driving the extrusion of a series of DNA loops[98]. DNA **loop extrusion [G]** involving the binding of 'ring-shaped' SMC complexes to the DNA and their subsequent ATP hydrolysis-driven conformational changes to induce and processively enlarge DNA loops, is thought to be the main driver behind genome folding and chromosome architecture in both bacteria and eukaryotes[13–18,99–101], Although this model is now widely accepted, it has also been reported that DNA binding and crosslinking may also contribute to SMC protein function[102].

SMC complexes are responsible for lengthwise compaction of the *E. coli* genome by extruding DNA loops from an ~130 nm axial core consisting of MukBEF complexes[103] in a manner previously shown to be carried out by eukaryotic condensins (see later). This lengthwise compaction was shown to be dependent on the presence of MatP, which displaces MukBEF from the Ter macrodomain. Following MukBEF release, Ter displays unique structural features, with contacts being restricted to ~280 kb instead of the much longer-range Mb contacts found outside this macrodomain[103,104]. In the presence of MatP,

MukBEF-driven DNA compaction results in a more linear or C-shaped chromosome with its ends linked by the less condensed Ter domain, whereas in its absence, MukBEF coats the whole chromosome resulting in the formation of a more circular chromosome[103].

Single-molecule fluorescence microscopy experiments in *E. coli* showed that ~10 MukBEF complexes accumulate in a few distinct genomic regions to drive DNA organization in an ATP-dependent manner and that in total approximately 100 MukBEF complexes are present per cell[105]. By measuring the fraction of chromosome-bound MukBEF complexes combined with the aforementioned MukBEF numbers, one study determined that the ~130 nm axial core of the bacterial genome, contains one MukBEF complex per ~6 nm core length, thereby forming a dense MukBEF array with 20-50 kbp DNA loops projecting from it and resembling a 'bottle brush'[103] (**FIG. 3c**). Approximately 15 topoisomerase IV heterotetramers per cell (from a pool of ~60) were found to associate with the MukBEF clusters close to the Ori region[106], most likely via direct protein-protein interactions[107]. The type 2 topoisomerase IV both decatenates DNA and can remove both positive and negative DNA supercoils[90,108]. This topoisomerase IV most likely controls DNA entanglement levels whereas MukBEF arrays extrude DNA loops, aiding timely chromosome segregation.

In *B. subtilis*, the loading of SMC complexes is thought to occur with the help of the protein ParB at specific *parS* loading sites positioned close to the Ori[109]. These sites are absent from the *E. coli* genome. Hi-C approaches suggest that after loading, the SMC complexes start to processively extrude DNA loops, and that their continuous translocation along the DNA possibly 'zips up' the chromosome by holding the two arms together, thereby facilitating chromosome segregation[110–112] (**FIG. 3d**), followed by SMC unloading at the terminus region[113]. A recent study showed that SMC complexes in this system can bypass each other in vivo during loop extrusion[114]. By engineering defined *parS* SMC-loading sites in the genome, the authors developed a SMC complex 'crash-course track' system. In this way, SMC complexes were induced to run into each other, and corresponding changes in DNA organization and SMC-protein localization were read out using Hi-C and chromatin immuno-precipitation (**ChIP [G]**) sequencing assays,

respectively. The resulting Hi-C maps could only be explained if SMC complexes were allowed to bypass each other. In vivo, this would allow for the resolution of SMC traffic jams and efficient genome organization utilizing multiple *parS* loading sites.

In summary, it is now generally accepted that the combined activity of SMC complexes and topoisomerases organizes the *E. coli* genome into a 'bottle-brush' structure, with a series of spatially-isolated negatively supercoiled DNA loops emanating from a central scaffold, organized by nucleoid-associated proteins such as MukBEF, MatP and topoisomerase IV[9,10,63,83] (**FIG. 3b,c**). The plectoneme-based organization of the bottle-brush isolates small parts of the genome and thereby minimises entanglement of the DNA via **entropic repulsion [G]** of DNA loops[115]. This is vastly different from the homogenous DNA organization of bacteriophage DNA, where proteins are absent from the compacted DNA phase. Perhaps surprisingly, the bacterial bottle-brush organisation is analogous to the DNA organisation of vertebrate mitotic chromosomes (discussed below). The main differences are that in eukaryotes the DNA is confined within a nuclear compartment by a double membrane envelope during most of the cell cycle, that nucleosomes also contribute to DNA organization by increasing the compaction of the chromatin fiber and that prior to cell division the interphase chromatin undergoes a dramatic reorganization to form an array of nested chromatin loops gives rise to cylindrical mitotic chromosomes.

[H1] DNA organization in eukaryotes: a timeline of different motor activities

During interphase, eukaryotes segregate their genomic DNA from the cytoplasmic protein-synthetic machinery inside a specialized compartment, the nucleus. Within the nucleus, chromosomes tend to occupy individual territories (**FIG. 4a**), with the DNA of each chromosome organized at different hierarchical levels. At the highest level, interphase chromosomes consist of distinct A and B compartments (typically several Mb in size), which correspond to active (open) and inactive (closed) chromatin, respectively[116]. Interphase Hi-C contact maps therefore feature a characteristic

checkerboard pattern, arising from high intracompartmental and low intercompartmental DNA contact frequencies[116]. This pattern is lost after G_2 phase when cells enter mitosis (**FIG. 4b**). Further increases in the resolution of Hi-C has led to the identification of at least five subcompartments (two in compartment A; A1 and A2, and three in compartment B; B1, B2 and B3)[117]. Similar interphase contact maps can be obtained from high-resolution fluorescence microscopy data, with multiplexed error-robust fluorescence in situ hybridization (**MERFISH [G]**) being a particularly powerful imaging approach to visualize 3D DNA organization[118].

At the next lower level of organisation, topologically associated domains (**TADs [G]** , clusters of loops ~200 kb to 1 Mb in size) may help the cell to regulate the expression of specific sets of genes[119]. TADs are defined by specific domain boundaries at which the CCCTC-binding factor (CTCF) binds and recruits cohesin. A previous study used polymer simulations to show that (cohesin-based) loop extrusion within boundary elements is an efficient mechanism to drive the formation of TADs, recapitulating complex chromosome interaction patterns[120]. CTCF binding also insulates each domain from neighbouring TADs[121–123]. On shorter length-scales (~100 kb) networks of DNA looping interactions exist, often bringing together gene promoters and regulatory sites such as enhancers[124]. CTCF sites with **convergent orientation [G]** block and stabilize cohesin after loop extrusion bringing the two CTCF boundaries into contact at the base of a chromatin loop[117,125]. However, recent high-resolution live-cell imaging experiments and accompanying polymer simulations revealed that TADs might predominantly exist as partially formed DNA loops, bringing CTCF boundaries closer together but not necessarily in contact[126]. Furthermore, in vitro experiments have recently shown that the blocking of cohesin by CTCF is DNA-tension dependent[127].

During interphase, approximately ~160,000 cohesins are dynamically bound to chromatin with an additional ~90,000 free in the nucleoplasm of HeLa cells[128]. The same study estimated that about ~120,000 CTCF binding sites (with variable interspacing) and ~180,000 CTCF molecules are present per HeLa cell. These numbers are consistent with the number of cohesins given that not all may be positioned at cohesin/CTCF enrichment

sites. The loss of cohesin results in the loss of DNA looping interactions (e.g., TADs) and its subsequent reintroduction restores the DNA loops. Thus, cohesin is a dominant genome folder during interphase[129]. The precise mechanisms driving DNA organization during interphase are beyond the scope of this review but may involve several additional mechanisms in addition to loop extrusion. These include **phase separation [G]** and interactions with the nuclear lamina[130]. Readers are referred to several reviews on the topic for more information[14,125,131].

[H2] DNA loop extrusion by SMC complexes

Recently, a variety of elegant in vitro single-molecule assays have provided important insights into the dynamics of loop extrusion by various SMC protein complexes (**BOX 3**). Using a DNA-loop extrusion assay, it was shown that yeast condensin can extrude DNA loops in an ATP-dependent manner with rates up to ~1.5 kb/s[132]. This agrees remarkably well with the rate of DNA packaging by the T4 DNA packaging motor (~2 kb/s) and also with the rate of loop extrusion by condensin II measured in vertebrate cells, 0.5 - 3 kb/s[133]. However, the SMC motor is relatively weak, stalling at 1.2 ± 0.5 pN[132], almost 100 times weaker than the T4 DNA packaging motor (**FIG. 2c**). The assay involved tethering the ends of a piece of fluorescently labeled lambda DNA to the surface of a glass slide, followed by the addition of condensin and ATP (**FIG. 4c**). Initially, fluorescence intensity along the DNA is relatively homogenous, however, loop extrusion results in a local increase of fluorescence intensity, corresponding to loop formation (**FIG. 4c**). Changes in fluorescence intensity along the DNA scale with changes in DNA density, providing quantitative information about the loop extrusion process in real time. Surprisingly, these experiments revealed that condensin extrudes loops in an asymmetric fashion. However, it is possible that condensin might switch between one-sided and two-sided loop extrusion in vivo depending on how the SMC complex engages with the DNA and how changes in its structural conformation are coupled to extrusion[134,135]. Indeed, polymer simulations have now shown that one-sided loop extrusion is insufficient to drive complete chromosome compaction and that a population of two-sided extruders (not necessarily

symmetric) is required[136]. Furthermore, an apparent symmetric loop extrusion could actually reflect a one-sided loop extruder rapidly alternating between sides. This so-called "switching model" was found to be the only one-sided extruder model capable of achieving proper chromosome compaction[137]. Regulation of this behavior potentially allows toggling between one-sided and two-sided loop extrusion.

A year after the demonstration of loop extrusion by yeast condensin, a similar assay was used to show that human cohesin extrudes DNA loops in an ATP-dependent manner with rates up to ~2.1 kb/s, but only in the presence of its NIPBL-MAU2 loader[138]. In contrast to condensin, cohesin was found to extrude loops in symmetric fashion (also see[99]). In the same year, a paper described the same result but used DNA curtains (multiple pieces of flow-stretched DNA connected to the glass via only one end) instead as a DNA substrate[139]. In addition to uncovering ATP-dependent symmetric loop formation they found that cohesin can extrude loops containing nucleosomal DNA at extrusion rates (~0.5 kb/s) similar to those found with naked DNA. In vitro experiments on condensin and cohesin using immunodepleted *Xenopus* egg extracts have confirmed the above results and furthermore showed that the activity of the condensin and cohesin SMC motors is differentially regulated[140]. Recently, SMC5/6 was also found to be an ATP-dependent DNA loop extruder, extruding loops at ~1.1 kb/s in a predominantly symmetric fashion, similar to cohesin[141].

Most in vitro experiments investigating loop extrusion have been performed using naked lambda phage DNA (48.5 kb). This is useful, as it is a well-defined template of appropriate size that allows for straightforward comparison between different in vitro experiments. In vivo, however, SMC complexes must perform their activities on chromatin, which is highly dense, locally supercoiled, and occupied by protein complexes such as nucleosomes and transcription machinery. This therefore provides SMC complexes with a much more challenging task[15]. It therefore remains to be seen how the in vitro results translate to the DNA packaging of chromatin in the crowded and complex environment of the cell. To try to reduce this gap in knowledge, in vitro experiments can be increased in their complexity, although this is challenging. For example, one study

increased the number of condensins acting on the DNA in vitro and showed that multiple condensins extruding loops from the same piece of DNA affect each other's loop extrusion dynamics[142]. Furthermore, condensins were found to bypass one another to form more complex loops, called "Z-loops", which is another mechanism that could turn two initial one-sided extruders into a combined two-sided DNA loop extruder[142].

By inducing DNA supercoils using the DNA intercalating dye SYTOX Orange, it was shown that condensin preferentially binds to the tip of plectonemes (see DNA supercoiling section) and can recruit and merge adjacent plectonemes to generate large stable supercoiled loops[143]. In this respect, the association between topoisomerase IIα and condensin in vivo is highly interesting (see below). Finally, by introducing roadblocks of different sizes (nucleosomes, RNA polymerase and dCas9) one study showed that SMC proteins can bypass these objects during loop extrusion. Most surprisingly, much larger roadblocks (such as 200 nm gold particles), could also be bypassed by condensin and cohesin[144]. Furthermore, simulations reveal that during mitotic chromosome formation, condensins must bypass cohesive cohesin holding sister chromatids together in order to generate chromosomes with two individual chromatid axes[133]. In doing so they effectively bypass the entire other sister chromatid. These data are inconsistent with the original view that loop extrusion by SMC proteins involves movement of the DNA through a topological ~50 nm loop created by the paired coiled-coils of the two SMC subunits of each complex[14] (**BOX 3**). The mechanism of loop extrusion[18], which was thought to be largely understood, has now become a mystery once again.

[H2] DNA organization of mitotic chromosomes

During mitosis, the DNA architecture of chromosomes changes dramatically to facilitate segregation of the sister chromatids[145]. During mitotic chromosome formation, the characteristic Hi-C checkerboard pattern that exists during interphase is lost, reflecting the loss of the interphase compartments and TADs[146]. Instead, a widening diagonal band can be observed reflecting a more homogeneous and sequence-independent type of

DNA organization (**FIG. 4b**), closely resembling the architecture of a DNA 'bottle brush' (**FIG. 3c, 4d**). That human mitotic chromosomes might be organized as DNA loops around a central scaffold was first proposed by Paulson and Laemmli, who visualized histone-depleted HeLa chromosomes using electron microscopy[147]. These chromosomes were depleted of most of their histone and nonhistone proteins after mitotic chromosome formation. More recently, researchers have added mouse sperm DNA to Asf1-depleted *Xenopus* egg extracts to show that structures resembling mitotic chromosomes can form in the absence of nucleosomes, consistent with condensin as a key driver of mitotic chromosome formation[148]. Indeed, several theoretical analyses have now shown that DNA loop extrusion is an efficient mechanism to drive the compaction of DNA and to form rod-shaped mitotic chromosomes[15,16,101,136,149,150]. An important remaining question is how the loops of mitotic chromosomes are arranged in vivo.

A study published in 2018, substantially improved our understanding of the process of mitotic chromosome formation by utilizing chicken DT40 cell cultures, which can be highly synchronized by arresting cells at the brink of mitotic entry, in late G_2 phase[151]. Release from Cdk1 inhibition is rapidly followed by mitotic chromosome formation, and was studied at 2.5 min time resolution using fluorescence microscopy and Hi-C. The Hi-C maps revealed that within 5 min (that is, by late prophase), compartments and TADs had mostly disappeared, reflecting the rapid reorganization of the chromosomal DNA. Strikingly, after 15 min (in prometaphase) a second diagonal band appeared in the heat maps (**FIG. 4b**). This moved away from the centre diagonal during chromosome formation, reflecting a dynamic change in sequence non-specific DNA interactions over the range from ~3 Mb to ~12 Mb across the whole chromosome, with the exception of the centromere. Polymer modeling revealed that this reflected an overall helical trend in the organisation of the chromatin loops, with an initial pitch of 3 Mb/turn (comprising 40 DNA loops) evolving into one of 12 Mb/turn (comprising 150 DNA loops with a thickness of ~200 nm). Light microscopy and modelling suggested that these loops were organised around a central condensin spiral staircase-like scaffold.

Depletion of condensin I (which is cytoplasmic in interphase and does not act in the initial steps of chromosome formation) and condensin II (which is nuclear in interphase) using an auxin-based degradation system, revealed that they have distinct roles. Condensin II acts during prophase to extrude large stable loops up to a size of several hundred kb that follow a disordered helical path along each chromatid. Condensin I subsequently binds within these large condensin II loops at the onset of prometaphase, extruding nested ~80 kb loops that further drive chromosome compaction. Quantitative fluorescence microscopy in HeLa cells subsequently revealed that mitotic chromosome formation involves the initial, more stable localization (>5 min residence time) of ~35,000 condensin II motors and the subsequent more dynamic localization (~2 min residence time) of ~195,000 condensin I motors[152].

Chromosome formation requires the activity of the two different condensin complexes to drive DNA loop extrusion. However, condensins are not the only proteins involved. Three other proteins also actively contribute to chromosome formation, namely the chromokinesin KIF4, topoisomerase IIα and cohesin. Fluorescence microscopy experiments have shown that condensin localizes along the chromosome axis together with topoisomerase IIα and KIF4[153,154]. Briefly, condensin can directly interact with topoisomerase IIα, and this could relieve condensin-induced supercoiling of DNA[143,155,156]. Furthermore, topoisomerase IIα is crucial to resolve unwanted DNA knots and entanglements during mitotic chromosome formation and throughout mitosis[13]. Its depletion in prometaphase using auxin-based degradation was shown to increase the amount of ultrafine DNA bridges in mitosis and also affect chromosome morphology[157]. The role of the KIF4 motor is more elusive, but the chromokinesin is known to bind DNA and to directly recruit condensin I to chromosomes[154,158,159]. Paradoxically, the roles of KIF4A in chromosome structure require a functional kinesin motor domain, but not microtubules[154,159]. Given its interaction with condensin and its multiple DNA binding domains, it is possible that dimeric KIF4 motor proteins bring multiple condensins together to coordinate loop extrusion, stabilize DNA loops after having been extruded by condensin, or bridge and stabilize DNA loops by itself.

It has been widely assumed that the only role of cohesin in mitotic chromosome structure and function is to maintain sister chromatid pairing at centromeres, as most cohesin is lost via the prophase pathway[160,161]. However, very recent studies have revealed that significant amounts of cohesive cohesin remain along chromosome arms well into prometaphase in chicken DT40 cells[133]. This cohesive cohesin plays an important role during mitotic chromosome formation, probably by constraining the ability of sister chromatids to fold autonomously during condensin loop extrusion.

In order to distinguish between the roles of condensin I and II in chromosome formation a recent study performed Hi-C, light and electron microscope imaging and polymer modeling of chromosomes formed in the presence of only condensin II, only condensin I or both condensins, all in cells lacking cohesin[133]. Chromosomes formed only by condensin I are highly irregular in their morphology and are best modelled as arrays of loops that follow a stretched random coil path (**FIG. 4g**). In contrast, chromosomes formed from only condensin II are cylindrical, but lack any precise reproducible internal structure (**FIG. 4f**). Instead, they consist of an ensemble of stochastic loops arrayed from one end of the chromatid to the other, and exhibiting a weak and disorderly helical trend. The condensin II scaffold is a discontinuous and disordered structure that is scattered throughout the interior of the chromatid. Chromosomes containing both condensins can be modeled by combining/overlaying the loop arrays from the two individual models retaining their original parameters, suggesting that their effects on chromosome formation are essentially additive (**FIG. 4e**). These data reveal that mitotic chromosomes are ensembles of disorderly structures, thus accounting for the challenges they have posed as researchers have sought to solve the mystery of how they are formed.

Formation of mitotic chromosomes is a complex process involving many different players that act at different points in time[162]. We are only beginning to understand the interplay between these different components and how they drive the DNA organizational changes required. For more comprehensive reviews on mitotic chromosome formation see[13,163] and on chromokinesin see[164,165].

[H1] Conclusions

Over the past decade, a combination of technological breakthroughs has accelerated our understanding of genome organization and the mechanisms involved. In vitro assays have become powerful tools to mechanically dissect DNA motor function and their ever-increasing sophistication (for example using DNA networks and multiple organizers) holds great promise. At the same time, increasing resolution of fluorescence microscopy-based methods and sequencing-based approaches have allowed for dissection of DNA network organization at shorter length scales, in vivo. Moreover, the precise manipulation of interphase DNA[166,167] or mitotic chromosomes[166,168] coupled to high-resolution force-displacement readouts are emerging methods that will further increase our mechanical understanding of the properties of the genome and the motor proteins that organisms use to package it.

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Display Items

Figure 1 | Bacteriophages package their DNA using a single molecular motor. A, T4, lambda (λ) and P22 bacteriophages displayed to scale. Scale bar, ~50 nm. The T4 bacteriophage head contains a relatively large 168.9 kb dsDNA genome[54], organized as a toroid oriented parallel to the T4 long axis (panels **Aa**, **Ad** and **d**). The 925 Å long contractile tail of T4 ends with a complex baseplate from which 6 long tail fibers extend[41,169]. The head of bacteriophage lambda (panel **Ab**) contains 48.5 kb dsDNA, spooled in perpendicular orientation in respect to the axis of its 135 nm long tail[1,170]. The head of P22 bacteriophage (panel **Ac**) contains 41.7 kb of DNA[171]; its tail is non contractile with a single long tail fibre. **B,** T2 bacteriophage with giant loops of DNA emerging from its head after lysis induced via osmotic shock and mounted using Kleinschmidt spreading (**Ba**). Scale bar, 1 μm; scale bar, 0.1 μm (**Bb**). Image adapted from ref[33]. **C,** Model showing the hexagonal arrangement and spacing of concentric layers of DNA in the P22 bacteriophage head. Adapted from ref[1]. **D,** Model showing the toroidal DNA organization in T4 head. Outer DNA layers are packaged first with DNA loops oriented parallel to the T4 longitudinal axis. Adapted from ref[41]. **E,** The P22 portal protein (side view [**Ea**] and top view [**Eb**]) packages the DNA via its central DNA channel (top view). Structure (PBD: 5JJ1) adapted from ref[172]. **F,** The portal ring forms the basis of viral head assembly. After capsid assembly, the motor complex docks the portal ring to start the DNA-packaging process. Once DNA packaging is complete, the terminases of the motor complex undock and tail formation completes the process. Adapted from ref[3].

Figure 2 | Properties of DNA packaging and DNA interacting proteins across scales. **a,** Plot of the packaging rate versus force exerted by the ϕ29 bacteriophage motor during DNA packaging. A progressive increase in DNA density inside the bacteriophage head leads to a decreased DNA packaging rate and increased motor load force. Graph reproduced from[51]. **b,** Internal forces inside the ϕ29 bacteriophage head that resist packaging (such as electrostatic repulsion and DNA bending) increase with the percentage of its 19.3 kb genome[55] that is packaged. Graph reproduced from[51]. **c,**

Structural maintenance of chromosomes (SMC) complexes can extrude DNA at high rates but have a relatively low stall force compared to bacteriophage motor complexes. Dynein and Ndc80 complex, which couple chromosomes to microtubules, have moderate stall force or rupture force, respectively. The bacterial FtsK packaging motor, which coordinates cell division and chromosome segregation in *E. coli*, can translocate at extremely high rate and has a high stall force. Data from refs[51,53,110,133,134,139–141,173–176].

Figure 3 | Organizational features of the bacterial genome are driven by DNA-associated proteins. a, Transmission electron microscopy (TEM) image of a bacterial chromosome 3 min after spreading, revealing extensive supercoiling of DNA loops surrounding an electron-dense core. Scale bar, 1 μm. Adapted from ref[66]. **b,** Chromosome organization in *E. coli*, *B. subtilis* and *C. crescentus* is species and cell-cycle specific (examples are from specific cell-cycle states, see ref[11]). In *E. coli*, MatP specifically organizes the DNA of the 800-kb-long Terminus (Ter) region (grey) via bridging of its 23 *matS* motifs, and loading of structural maintenance of chromosomes (SMC) complexes (arrows) does not depend on *parS*. Two non-structured regions (NSR) flank the Origin (Ori) region. In *B. subtilis*, loading of SMC complexes occurs at *parS* sites located close to the Ori region. In *C. crescentus*, the Left and Right macrodomains run along the longitudinal axis of the bacterium. DNA plectonemes are an organizational hallmark of the bacterial genome. Adapted from refs[9,11]. **c,** Characteristic 'bottle-brush' organization of the bacterial DNA. Nucleoid-associated proteins and SMC complexes organize the bacterial genome. Adapted from ref[63].**d,** Normalized Hi-C interaction maps showing the 'zipping' of the 4.2 Mb *B. subtilis* genome by SMC complexes, which are loaded at a single *parS* site. Image courtesy of David Rudner and Xindan Wang. Adapted from ref[110]. SMC complexes are depicted here engaging with the DNA in a topological manner, however, the precise SMC structural conformation as well as the position of DNA during SMC-dependent DNA movement remain unclear.

Figure 4 | The eukaryotic genome is hierarchically organized by SMC proteins. a, (upper) Chromosomes occupy distinct territories inside the nucleus during interphase, here imaged using fluorescence microscopy and 3D-FISH with chromosome 1 and 20 visualised in preserved fibroblast nuclei. (lower) The same chromosomes in a mitotic chromosome spread. Scale bar, 5 μm. Image reproduced from ref[23]. **b,** Bursal lymphoma chicken cells (DT40) carrying an analog-sensitive CDK1 mutation (CDK1^{as}) were synchronized and harvested at time points indicated. Corresponding Hi-C contact frequency heatmaps reveal the dramatic reorganization of the DNA during mitotic chromosome formation. Image courtesy of Johan Gibcus and Kumiko Samejima (data unpublished). **c,** Fluorescence images showing loop extrusion of fluorescently labeled lambda DNA (48.5 kb long, sytox orange [SxO] labeled) by condensin in vitro. Constant buffer flow limits the tethered DNA and the extruded DNA loop to the image plane. Arrow (white) indicates initial loop formation, with the loop appearing white due to higher DNA density. SMC, structural maintenance of chromosomes proteins. Scale bar, 2 μm. Image data courtesy of Cees Dekker Lab, corresponding to ref[144]. **d,** Chromosome isolated from HeLa cells deposited on electron microscopy (EM) grid and swollen using low ionic strength buffer. Extensive DNA loops can be seen emerging from the chromosome arms. Dots along these loops correspond to nucleosomes (also see inset, region 2x magnified). Scale bar, 1 μm. Image reproduced from ref[177]. **e,** Chromosome compaction by condensin II and I (no cohesin), modeled as a series of nested loops packed as a disordered helix (a helical trend) in a cylindrical volume with the characteristic shape, dimensions and density of the mitotic chromosome. **f,** Chromosome compaction by condensin II only modelled as a series of large loops organized as a disordered helical cylinder that is wider and shorter than a corresponding wild-type chromosome. **g,** Chromosome compaction by condensin I only is best explained by a series of small loops organized as an extended random walk, lacking the characteristic cylindrical shape of a wild-type mitotic chromosome. Panels e-g adapted from ref[133] and courtesy of Anton Goloborodko prior to publication. SMC complexes are depicted engaging with the DNA in a topological manner, however, the precise SMC structural conformation as well as the position of DNA during SMC-dependent DNA movement remain unclear.

Box 1. Optical Tweezers

In 1970, Arthur Ashkin discovered that transparent microspheres floating in suspension could be trapped and manipulated using a tightly focused laser beam[178]. In brief, the transfer of momentum from the refracted photons to the microsphere keeps the sphere stably positioned close to the center of the trap, where gradient and scattering forces are balanced[179] (**Panel A**). Displacement of the sphere from the center of the trap is directly correlated to the amount of external force applied (**Panel B**), and conveniently overlaps with the biologically relevant force range of 0.1-100 pN. This force-dependent displacement can be read-out at very high spatiotemporal resolution; optical traps can detect sub-pN changes in force or sub-nm changes in displacement with sub-ms time resolution, depending on the 'stiffness' of the trap (0.1-0.3 pN/nm per 100 mW of trap power[179]). This makes optical tweezers excellent instruments to study force-dependent molecular properties and to unravel conformation changes of macromolecules (**Panel C**). Since optical tweezers are normally combined with sophisticated flow chambers, protein density can be easily controlled, providing straightforward access to single-molecule measurements. In this way, the stochastic mechanochemical behavior of single DNA-associated motor proteins can be monitored in real time. Furthermore, optical tweezers can be combined with fluorescence microscopy to directly visualize DNA-protein complexes as well as their DNA substrate during force-displacement measurements[180]. (Figure panels adapted from ref[179]). In the future, it is likely that optical tweezers will have a pivotal role in dissecting the activity and associated conformational changes of SMC complexes as well as that of other DNA-associated proteins during DNA reorganisation.

[BOX 2] Hi-C and Hi-C Data Analysis

Determining the detailed 3D organisation of chromosomes is challenging due to high DNA density and the vast amounts of DNA present in cells. In 2002, a landmark paper introduced a new technique termed chromosome conformation capture (3C) to obtain high-resolution structural information of chromosomes[181]. In 3C, nuclei are isolated and

fixed, followed by DNA digestion and ligation of crosslinked DNA fragments. The DNA ligation reaction is performed at low DNA concentration, so that the joining of two crosslinked DNA fragments (assumed to be in close proximity in the cell) occurs with vastly higher frequency compared to ligating two non-crosslinked DNA fragments. After the ligation step, crosslinks are dissolved, and the ligation products are identified and quantified using qPCR with primers specific for pairs of selected loci. DNA crosslinking probability directly correlates with DNA-DNA interaction frequency, which depends on the DNA-DNA proximity and flexibility of the DNA strands.

The introduction of Hi-C in 2009 dramatically improved the impact of the 3C approach, by combining it with massive parallel sequencing instead of predetermined (locus-specific) primers[116]. In brief, Hi-C is similar to 3C, except that DNA fragments are biotinylated preceding DNA ligation (see Figure). Subsequent shearing of the DNA allows for purification and enrichment of crosslinked DNA fragments and the generation of a Hi-C library. Sequencing of the library identifies the galaxy of interaction pairs, which are then aligned to the relevant genome to retrieve positional information. DNA crosslinking frequencies are most often converted into an interaction or frequency matrix, which can be displayed as a spatial heatmap. Polymer modeling can be used to determine the 3D structure that most accurately reflects the heatmap or frequency matrix using the sequence-related contact frequencies. (Figure adapted from ref[10]). As Hi-C can be performed on massive scale, it facilitated construction of the first maps of the interchromosomal organization covering whole genomes[182].

[BOX 3] SMC Complexes

Structural maintenance of chromosomes (SMC) protein complexes are a class of DNA-associated motor proteins responsible for the structural (re)organization of DNA[9,17,18]. These proteins were first identified in a screen for **s**tability of **m**ini**c**hromosomes, but were soon re-branded as **s**tructural **m**aintenance of **c**hromosomes when it was realized that they act across many species to regulate chromosome transactions. Surprisingly,

analysis of the sequence of these proteins revealed a striking similarity to ABC ATPases, which at that time were primarily known to be involved in membrane transport[183]. Even more surprisingly, the two structural motifs (Walker A and B, which combined form the **A**TP-**b**inding **c**assette (ABC – Walker A recruits the ATP molecule, whereas Walker B coordinates the Mg^{2+} to catalyze the ATP hydrolysis reaction) were located at opposite ends of the molecule, separated by two large regions of coiled-coil flanking a central hinge. (SMC mechanochemical cycle adapted from ref[23] and SMC structure adapted from ref[184] (PDB: 6YVU)).

Eukaryotic SMC proteins form three different classes of protein complexes: condensin I & II, cohesin and SMC5/6[17,185]. Each class has a single pair of SMC proteins, a single kleisin (linker) protein and varying numbers of auxilliary subunits, which are often HEAT repeat proteins. The SMC protein pairs are SMC1/SMC3 for cohesin, SMC2/SMC4 for both condensins and SMC5/SMC6 for the epinonymus SMC5/6 complex.

A characteristic feature of ABC ATPases is that the Walker A and B sites only dock to one another in the presence of bound ATP. The peculiar distribution of those sites in SMC proteins means that the Walker A and B sites can only come in contact if the molecule forms a jacknife fold or forms a head-to-tail dimer. In fact, the jacknife fold is the solution adopted by all SMC proteins, but with an unexpected twist. Thus, in the presence of ATP, the Walker A site of SMC2 docks with the Walker B site of SMC4 (and vice versa) (see Figure). The hinge domains of the SMC pairs also dock to one another constitutively, so that ATP-binding and hydrolysis opens and closes a ring that can be linked by the strap-like kleisin subunit creating several topological compartments[135,184]. These cycles of head docking and separation are linked to conformational changes of the coiled coils, which can either form a ring, a straight closed rod, or a rod with a scorpion-like fold-back[17]. These dynamics are linked to the ability of the SMC proteins to translocate DNA with a remarkable ~200 bp median step size, substantially larger than the ~50 nm size of the yeast condensin molecule. Indeed, steps of up to >500 bp (>170 nm) have been observed[186]. How the mechanochemical cycle of SMC complexes is linked to DNA translocation is currently an active area of research[17,18,99,100,187–190].

Glossary Terms

Lengthwise compaction | An overestimate of the true compaction ratio obtained by dividing the DNA contour length (its maximum linear extension) by the length of the major axis of the enclosing compartment.

DNA compaction | The reduction of the volume occupied by DNA or chromatin, which in eukaryotes, might be expected to be driven by changes in histone post-translational modifications.

Chromosome condensation | The re-organisation of chromatin that accompanies the disassembly of interphase chromatin structures and formation of compact mitotic chromosomes.

Contour length | The contour length of a (DNA) polymer is its length measured when fully extended, a condition that never occurs in living cells.

Fourier shell model | Spatial frequency analysis of the diffraction patterns to determine spherical shell spacing.

Molecular dynamics | Computational technique to capture the positioning of a set of molecules over time.

Stall force | The opposing force at which a motor protein stops moving or translocating cargo (in this case DNA or chromatin).

Dwell phase | The time that a motor protein is waiting, and no ATP-driven conformational changes are occurring.

Burst phase | The time that a motor protein is active, during which ATP-hydrolysis drives a series of conformational changes.

Nucleoid | Region of the bacterial cell containing the prokaryotic chromosome composed of DNA and associated proteins.

FISH | **f**luorescence **i**n **s**itu **h**ybridization: A fluorescence-microscopy approach that uses fluorescent sequence-specific adapters to visualize the chromosome at a specific genomic location.

Macrodomains | Mb-sized chromosomal regions that are spatially isolated.

DNA plectoneme | An extended structure in which the DNA double helix is wrapped around itself as a result of DNA supercoiling.

DNA supercoiling | The over- or under-winding of DNA

Loop extrusion | The SMC-driven formation of a DNA loop, which involves incorporation of adjacent DNA into a loop while the two ends are kept together at the base.

ChIP (Chromatin ImmunoPrecipitation) | A technique in which antibodies are used to pull down target proteins that are cross-linked to the DNA. Sequencing is then used to identify associated (genomic) regions.

Entropic repulsion | A force emerging from the fact that overlap of DNA loops is energetically unfavourable, preventing DNA entanglement.

MERFISH | **m**ultiplexed **e**rror-**r**obust **f**luorescence **i**n **s**itu **h**ybridization: A fluorescence-microscopy approach that builds up a structural map of the DNA, using the localization of large numbers of fluorescent sequence-specific adapters that are sequentially added, imaged and removed over time.

TADs | Topologically associated domains. Regions (typically encompassing 10^5 - 10^6 base pairs) within chromosomal territories that display high interaction frequencies (and insulation from neighbouring regions) within boundaries defined by binding of the protein CTCF to DNA target sequences.

Convergent orientation | Two CTCF binding sites facing each other, so that continuous loop extrusion brings them together at the base of the chromatin loop.

Phase separation | The emergence of two or more separate phases from a mixture such as the cytoplasm.

Table of Contents (ToC) blurb (~40 words)

In this Review, the authors summarize DNA packaging in bacteriophage, bacteria, and eukaryotic cells. They describe the difficulties each system faces when packaging its DNA, outline the molecular motor components involved, and provide insights from new studies that reveal how DNA organization is achieved.