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Historical perspective

Nucleosome dynamics: Sequence matters

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ABSTRACT

About three quarter of all eukaryotic DNA is wrapped around protein cylinders, forming nucleosomes. Even though the histone proteins that make up the core of nucleosomes are highly conserved in evolution, nucleosomes can be very different from each other due to posttranslational modifications of the histones. Another crucial factor in making nucleosomes unique has so far been underappreciated: the sequence of their DNA. This review provides an overview of the experimental and theoretical progress that increasingly points to the importance of the nucleosomal base pair sequence. Specifically, we discuss the role of the underlying base pair sequence in nucleosome positioning, sliding, breathing, force-induced unwrapping, dissociation and partial assembly and also how the sequence can influence higher-order structures. A new view emerges: the physical properties of nucleosomes, especially their dynamical properties, are determined to a large extent by the mechanical properties of their DNA, which in turn depends on DNA sequence.

Contents

1. Introduction

The genetic blueprint of all organisms is maintained in their DNA, consisting of sequences of billions of base pairs and encoding all the proteins that give shape and function to the cell. With modern high-throughput sequencing techniques, it has become routine to accurately map the base sequence of entire genomes. This has revolutionized our understanding of genetics. It has become abundantly clear though that the DNA sequence harbors many more features than protein code alone; only 2% of the human genome encodes proteins. However, 80% of it can be related to biochemical functions [1]. A better understanding of the essential processes that orchestrate life requires insight in those features, which go well beyond the central dogma of molecular biology.

While being a carrier of genetic information, DNA is a physical object that has mechanical properties and occupies space in the cell. Under physiological conditions, DNA generally assumes a right handed helix, with a rise of 0.34 nm per base pair (bp) and a diameter of 2 nm, known as B-DNA. The mechanics of B-DNA can be well captured by bend, twist, and stretch moduli, and classical and statistical physics provide the means to quantitatively describe the mechanics of generic DNA [2], typically averaging out all sequence related features. Modern single-molecule manipulation techniques have experimentally tested and refined this insight into DNA mechanics [3].

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As genomes can range up to several gigabase pairs, DNA is one of the biggest polymers in the cell. Given the rather high stiffness of B-DNA, genomes would occupy volumes much bigger than the cell itself if DNA would take the structure of a random coil. Instead, nature has evolved complex structures of DNA and proteins, chromatin, which condense the genome by several orders of magnitude. In eukaryotes, the smallest structural unit is a nucleosome, containing 147 bp of DNA wrapped around a core of 8 histone proteins in 1.7 turns of a left-handed super helix [4]. Repeats of nucleosomes, about every 200 bp [5], and complemented with linker histones and many other factors, arrange the genome in a dynamic, but condensed structure that is actively maintained by the cell.

While efficient in condensing the DNA, chromatin provides physical barriers that limit access to DNA by proteins that are essential for processes like transcription, replication, and repair. As outlined in this review in more detail, nucleosomes themselves are very dynamic structures. The opening and closing of nucleosomes, referred to as nucleosome breathing, as well as displacement, assembly and disassembly allow for seemingly unrestricted access to DNA, despite three quarters of our genome being tightly wrapped in nucleosomes.

As for DNA alone, some of the structural properties of chromatin can be readily captured in physical models. The higher complexity, both in shape and in composition, as well the limited availability of sufficiently detailed structural data, provide a great challenge though. About 40 years after the first reports on chromatin structure [6], the structure and dynamics of chromatin is still a very active area of research. Cryo-EM and X-ray scattering studies of chromatin from eukaryotic cells generally challenge the existence of chromatin organization into regular 30 nm fibers *in vivo* [7], a structure that is readily obtained in vitro [8]. Recent studies using advanced chromatin capture techniques [9], as well as high-resolution microscopy results [10], indicate the existence of larger (>kbp) chromatin domains that can be described in a statistical manner. Overall, these advances point to a rather irregular structure of chromatin *in vivo* that can only be described in statistical terms.

Many activities on DNA, however, with transcription as the prime example, are deterministic processes that appear to be carefully regulated by the cell. Not only is the timing of the transcription of specific genes of vital importance for the cell, it also requires the presence of transcription factors and the transcription machinery itself to be at the exact base pair location within the genome. This warrants a much more detailed look at chromatin structure and dynamics that goes beyond what can be captured typically by statistical models. Structural biology has provided ample examples of how biological function can be understood in terms of molecular structures that have been determined with Å resolution. Alas, the nucleosome appears to be very dynamic, and the techniques in use, like crystallography, EM tomography, and NMR, can only capture this to a limited extent.

Here, we review some of the excellent molecular biology, physical modeling, and single-molecule detection and manipulation studies on nucleosomes in the context of the role that DNA sequence plays in nucleosome structure and dynamics. As has been our own practice, many of these studies have generally ignored the role of DNA sequence in chromatin organization, assuming that the particular structures are representative for canonical DNA and nucleosomes. Often, the reason for this neglect is the need for sufficiently homogeneous and/or stable nucleosomes for such studies. In physical modeling, the complexity rises rapidly when refinements up to the base pair level are needed. However, with all exciting progress in this field, we feel that now it is opportune to look back and to evaluate to what extent DNA sequence affected reported results and to look forward to methods that can address these questions. This should lead to a thorough understanding of the structure and function of our genome.

The rapid advances in epi-genomics further exemplify that detailed mapping of the compositional heterogeneity of chromatin, down to single-nucleosome resolution, is instrumental in shedding light on many hitherto obscured processes, often with direct clinical relevance. A structural framework for the interpretation of these features, which is typically lacking, would further boost the impact of this knowledge. Nowadays, we are well aware that epigenetic modifications to the histone tails, the histone cores, as well as the DNA itself, can play a leading role in the regulation of processes like transcription, ultimately through changes in the structure of the nucleosomes. These could provide either a recognition site for factors downstream of the regulation pathway, and/or they could change features like nucleosome breathing, (dis-)assembly, and higher-order folding and thus directly modulate DNA accessibility. Either way, such changes will act in concert with differences in DNA sequence of the involved nucleosomes.

In this review, we will first discuss briefly some of the crystal structures of nucleosomes and the sequence related features that can be found in these structures. Closely related, there have been many efforts in predicting nucleosome positions on DNA. Next, we will discuss one of the most abundant structural changes in the nucleosome, nucleosome breathing, followed by forced nucleosome unfolding and nucleosome sliding. It is easily overlooked that nucleosomes can also partially assemble and disassemble, which will be discussed in another section. We then continue with some considerations on the interactions between nucleosomes that drive higher-order folding. In all instances, there is a role for DNA sequence. We finally discuss some theoretical approaches to deal with sequence effects in nucleosomes before we provide a tentative conclusion. It will be interesting to see in future studies if it is possible to fully understand how this sequence affects nucleosome structure, and perhaps most intriguingly, to determine whether nature makes use of these features to regulate processes like transcription, as has been hotly debated since the hypothesis of the nucleosomal code was raised [11].

2. Nucleosome structure

The core of the nucleosome particle consists of 8 subunits of four types of histone proteins, H2A, H2B, H3, and H4, which can interact with each other to form the heterodimers H2A–H2B and H3–H4 [4]. In the nucleosome, two H3–H4 dimers interact through a 4-helix bundle to create a tetramer. Binding of the tetramer with two H2A–H2B dimers via a similar mechanism leads to the formation of the histone octamer, which is stabilized by the attractive interactions with the DNA molecule. The nucleosome core particle manifests a two-fold symmetry, where the symmetry axis, i.e., the nucleosome dyad, passes through the central DNA base pair, see Fig. 1A and B.

Histone–DNA interactions mainly involve hydrogen binding between the negatively charged DNA phosphates and the positively charged elements at the surface of the octamer and are localized at 14 distinct binding sites where the minor groove of the DNA faces the octamer. In terms of the superhelical coordinate [4], which is defined by the number of DNA helical turns with respect to the central base pair, the binding sites occur at half-integer SHLs (SHL: superhelix location) from −6.5 to +6.5. Each nucleosome binding site mainly engages two consecutive “primary bound phosphates,” one at each of the DNA strands [12]. Each histone dimer in the nucleosome core provides 3 binding sites for the DNA molecule. These 12 binding sites are responsible for wrapping of 121 base pairs of the nucleosomal DNA and are divided into two categories [4]: the four binding sites at SHL +/−1.5 and +/−4.5 involve two alpha helices at the center of a histone dimer, one from each subunit, and thus are called α1α1 binding sites. The remaining binding sites at SHL +/−0.5, +/−2.5, +/−3.5, and +/−5.5 are L1L2 binding sites as they are formed by two adjacent loop structures, L1 and L2, at the ends of each histone dimer. The two outermost nucleosome binding sites at SHL +/−6.5 have a different nature and are formed by binding of the H3 N-terminal extensions to the 13 terminal base pairs at the two ends of the nucleosomal DNA. The binding sites over the H3–H4 tetramer are generally stronger than the

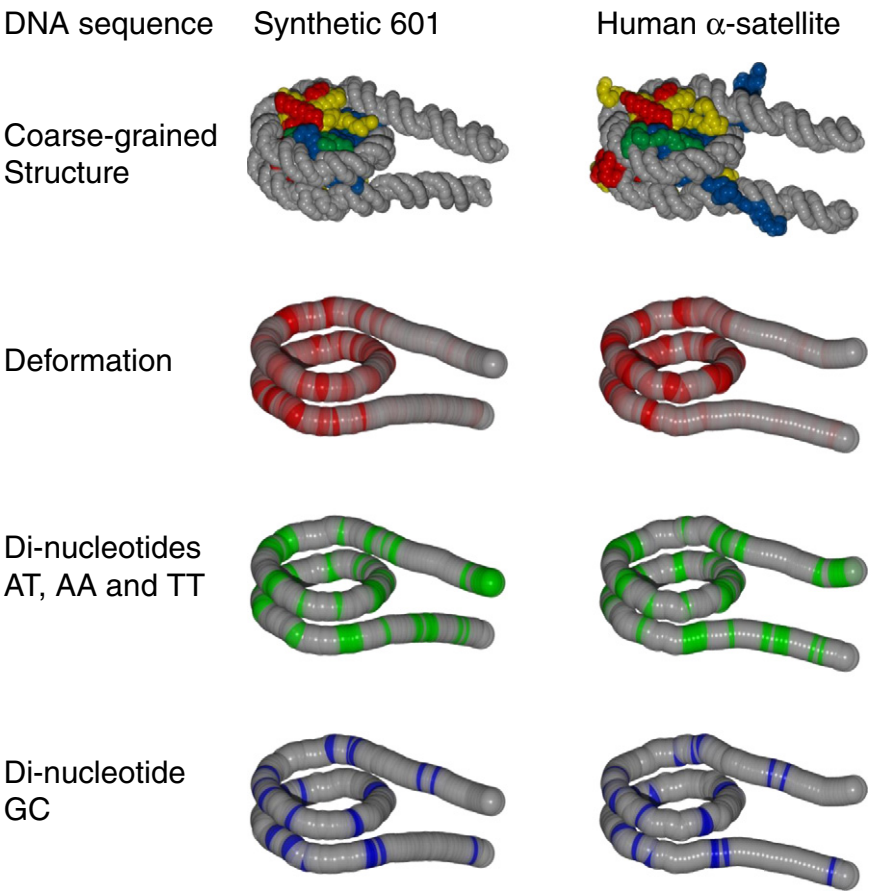

Fig. 1. Coarse-grained models of nucleosomes containing the synthetic 601 sequence, based on pdb 3LZ0 [16], and a 146 bp palindromic DNA sequence taken from one-half of a human α-satellite sequence repeat, based on pdb 1KX5 [167]. Top rows: Amino acids, sugars, phosphates, and bases are combined into a single 5 Å radius sphere. Gray DNA, yellow H2A, red H2B, blue H3, green H4. DNA was extended to 200 bp using a rise of 3.4 ± 0.1 Å, a roll of 0 ± 0.12 rad, and a twist of 0.60 ± 0.03 rad and a random DNA sequence. Standard deviations are based on the mean stretch, bend, and twist modulus of DNA. Rows 2–4: Further coarse graining to a single 10 Å radius sphere per base pair; proteins are omitted. Row 2: Red color shows deviations from the mean rise, roll, and twist, distributed along the DNA and weighted by one over the standard deviation of each parameter. In both nucleosome structures, there is a 10 bp periodic deformation along the superhelical path. Rows 3 and 4 show the distribution of AT, AA, and TT nucleotides in green and GC in blue, that largely correlate with stress points in the nucleosome, emphasizing the mechanical relation between nucleosome structure and DNA sequence.

ones associated with the H2A–H2B dimers [13] and thus deform the DNA more strongly [14].

The best fit of the nucleosomal DNA structure to a perfect superhelix has a radius of 41.9 Å and a pitch of 25.9 Å [12]. Interestingly though, the local curvature of the DNA is two times larger than the curvature of this ideal superhelix. A course-grained representation of the DNA at the base pair level [15] provides a detailed picture of DNA structure inside the nucleosome. In this course-grained scheme, 6 degrees of freedom are assigned to each DNA base pair step. Twist and rise degrees of freedom represent the rotation around and the translation along the direction perpendicular to the base pair surface, characterizing the helical structure of DNA. In addition, roll and shift correspond to the bending and shearing deformations in the local groove direction, and the corresponding deformations in the direction of DNA backbone are described by tilt and slide, respectively. Analyzing the crystal structure of nucleosome core particles with human α-satellite DNA sequence [4,12], as well as the strong nucleosome positioning 601 sequence [16], and their derivatives [14], have identified common features in the nucleosomal DNA structure at the base pair level. As expected, tilt and roll both show typical oscillatory trends, with a period equal to the DNA helical pitch and a phase difference of nearly 2.5 bp, which is an indication of the superhelical conformation of DNA in the nucleosome. However, while the tilt almost fully contributes to the formation of the DNA superhelix, the amplitude of roll oscillations is twice the expected value for the ideal superhelix, as visualized in Fig. 1C and D. This is the source of the observed excess curvature, which manifests itself as large negative rolls over the minor grooves (half-integer SHLs), and compensating large positive rolls at major groove regions (integer SHLs). The oscillation in roll is coupled to oscillations in twist and slide [12,14]. Major groove regions show systematic unwinding of the DNA accompanied by negative slide, while the opposite happens at the minor groove regions. DNA is generally more restrained in the minor groove regions [14]. Depending on the DNA sequence, the bending profile in these regions can be smooth, occasionally accompanied with large alternations in shift, or it can be concentrated in one base pair step to form a sharp kink [12].

Since the DNA molecule is significantly deformed in a nucleosome over a length nearly equal to its persistence length, and the local deformability of DNA changes with its sequence, one expects that the sequence of the DNA molecule affects both its affinity for nucleosome formation [17] and its structure inside the nucleosome [14]. Examples of these sequence-dependent effects are the appearance of highly flexible base pair steps such as TA (in 601 sequence) [14] or CA (in human α-satellite sequence) [12] at the center of the minor groove which have the strongest deformations, and the appearance of the motif TTAA in 601 sequence at SHL +/− 1.5, where an extreme narrowing of the minor groove is required [18]. The contribution of the correlated roll-slide oscillations in sequence-dependent nucleosome structure and nucleosome formation affinity has been highlighted in the literature [19]. The role of the alternating shift patterns at minor

groove regions, and their relevance to the GC content of the sequence, has also been appreciated recently [14] (Fig. 1E–H).

3. Nucleosome positioning

Even without high-resolution knowledge of the structure of the nucleosome, it was known that nucleosomes have a high preference for certain DNA sequences and tend to avoid other sequences [20]. Kaplan et al. concluded from genome-scale nucleosome mapping that intrinsic nucleosome sequence preferences have a dominant role in determining nucleosome organization *in vivo* [21]. Shortly after this study, Zhang et al. reported a similar study on *in vivo* and *in vitro* assembled yeast chromatin, but using a somewhat different methodology [22]. They and others [23] concluded that intrinsic histone–DNA interactions are not the major determinant of nucleosome positioning, but rather of nucleosome occupancy. The difference may be related to dynamic changes of the nucleosomes involved. More recently, nucleosome mapping was further improved [24,25] yielding single base pair accuracy.

Despite having this ultimate resolution, the discussion on the extent of the influence of DNA sequence on nucleosome positing has not settled yet. On the one hand, there are methodological limitations that may affect the outcome of these genome-wide studies. All methods require dissection of chromatin into single-nucleosome units, and both enzymatic and chemical methods for DNA cleavage may not be immune for sequence preferences and structural features of chromatin beyond the nucleosomes. Moreover, analysis and annotation of the large data sets often require thresholds and models that may affect the outcome. However, intrinsic variations in the composition depending on cell type, cell cycle, and environmental factors may play an even more important role. This was nicely demonstrated by Brown et al. [26], who pushed nucleosome mapping to single molecules of the Pho5 promoter using EM and could relate nucleosome occupancy of three distinct sites in the promoter region to transcription activity of the gene. Although the results of genome-wide and ensemble averaged nucleosome mapping provide less direct insight, it has become an important experimental tool for chromatin research. The outcomes of different studies are largely reproducible, pointing to a well-maintained distribution of nucleosomes along the sequence of our genome.

Based on the genome-wide maps of nucleosomes, that are now available for various organisms, there have been ample attempts to model and predict the nucleosome positions, solely based on DNA sequence [11,21,27,28,29]. Bioinformatics approaches typically use known nucleosome maps to learn statistical features that can then be applied for predicting nucleosome occupancy in other DNA sequences. Such analysis yields generic sequence rules, that largely align with known features of DNA in nucleosomes, such as avoiding A-tracts, and most importantly a strong preference for TA, TT, and AA dinucleotides to be positioned where the minor groove faces inwards and GC dinucleotides where the minor groove faces outwards (Fig. 1E–H). It should not be surprising that the resulting 10 bp periodicity of these dinucleotides can also be found in the strong nucleosome positioning sequences that are generally used for *in vitro* reconstitution of nucleosomes.

Alternatively, more physical approaches start with mechanical parameters of the nucleotides, which can be extracted from high-resolution diffraction and NMR structures of DNA and nucleosomes as well as Molecular Dynamics simulations [30]. These provide *ab initio* sequence rules for nucleosome positioning, leading to an energy landscape for nucleosomes along the DNA. However, other physical mechanisms, such as the statistical positioning of nucleosomes around inaccessible parts of the genome, being either very strongly positioned nucleosomes, transcription factors, the transcription machinery, and/or structural elements, also need to be included in the thermodynamics to properly account for densely packed chromatin fibers [31,32].

Some modesty is called for though: in a comparative study, Liu et al. [33] conclude that predictions of genome-wide nucleosome positions by all the tested methods perform only moderately better than random guess prediction. Moreover, accuracies gradually decrease from yeast to human, indicating that the physics governing nucleosome positioning on DNA is generic among species, but that other processes *in vivo* become increasingly important as genomes become larger. This should not be a surprise, given the high abundance of chromatin remodeling factors that actively displace nucleosomes and the presence of many other DNA binding proteins that compete with histones for a place on the heavily crowded DNA.

Chromatin reconstituted *in vitro*, from pure DNA and histones, better follows the models describing nucleosome positioning than chromatin assembled *in vivo* [21]. Surprisingly though, the locations of the strongest nucleosome positioning sequences, that are used to create well-defined chromatin structures, can generally not be resolved. Van der Heijden et al. [34] showed that for these selected sequences the position and the affinity of the nucleosome can be correctly calculated when the sequence constraints are only imposed on the central 70 bp of the nucleosome, rationalized by the stepwise assembly of first the tetramer and then the dimers during reconstitution. Although taking subassemblies and conformational dynamics, and their sequence dependence, into account will dramatically complicate our view on chromatin, there is substantial experimental and theoretical evidence that such an approach is needed to get a better grip on nucleosome positioning and other roles that nucleosomes play in DNA organization.

During the differentiation of a multicellular organism, specialized cells form, yet all the cells carry the same genome. Could sequence-dependent DNA mechanics play a role here? We know that differentiation is linked to epigenetic modifications [35]. One prominent example is CpG methylation. It is known that this chemical modification changes the mechanical properties of CG steps [30,36] and can cause changes in nucleosome occupancy [37,38]. How nucleosome positioning sequences are affected by CpG methylation however remains to be established. In that context, it is interesting to note that the only clear 10-base pair periodic signal for dinucleotide steps found in the human genome is CG [39].

4. Nucleosome breathing

Nucleosome breathing, or site exposure, is a mechanism where a stretch of DNA uncoils from one end of the nucleosome with the rest of the nucleosomal DNA staying wrapped, Fig. 2. This mechanism occurs spontaneously as the result of thermal fluctuations. Site exposure has been demonstrated first by Polach and Widom [40] (see also [41,42]) for a nucleosome that was bound to the positioning sequence of the sea urchin 5 S RNA gene, by adding restriction enzymes to a solution of nucleosomes. Different enzymes had their restriction sites buried at different depth inside the wrapped DNA. For an intact, fully wrapped nucleosome, the enzymes would not be able to bind to their sites. It was found that all enzymes could reach their target sites but that the equilibrium constant for site exposure decreases strongly toward the middle of the wrapped portion. A stepwise unpeeling mechanism was suggested as an explanation for the data (see also [43]).

Two experiments in 2000 by the Widom lab addressed the question whether changes to the nucleosome could lead to changes in site exposure. The first experiment [42] focused on epigentic modifications of core histone tails and tested whether lysine acetylations could have a substantial effect on site exposure. An upper bound of a possible impact of tail modifications was obtained by comparing the equilibrium constants for site exposure for tailless nucleosome to nucleosomes containing tails. A position-dependent 1.5- to 14-fold increase was found. It was concluded: “The smallness of the effect weighs against models of gene activation in which histone acetylation is a mandatory initial event, required to facilitate subsequent access of regulatory proteins to nucleosomal target sites.”

On the other hand, the second experiment [44] found a dramatic effect, a 10- to 100-fold suppression of site exposure, when something

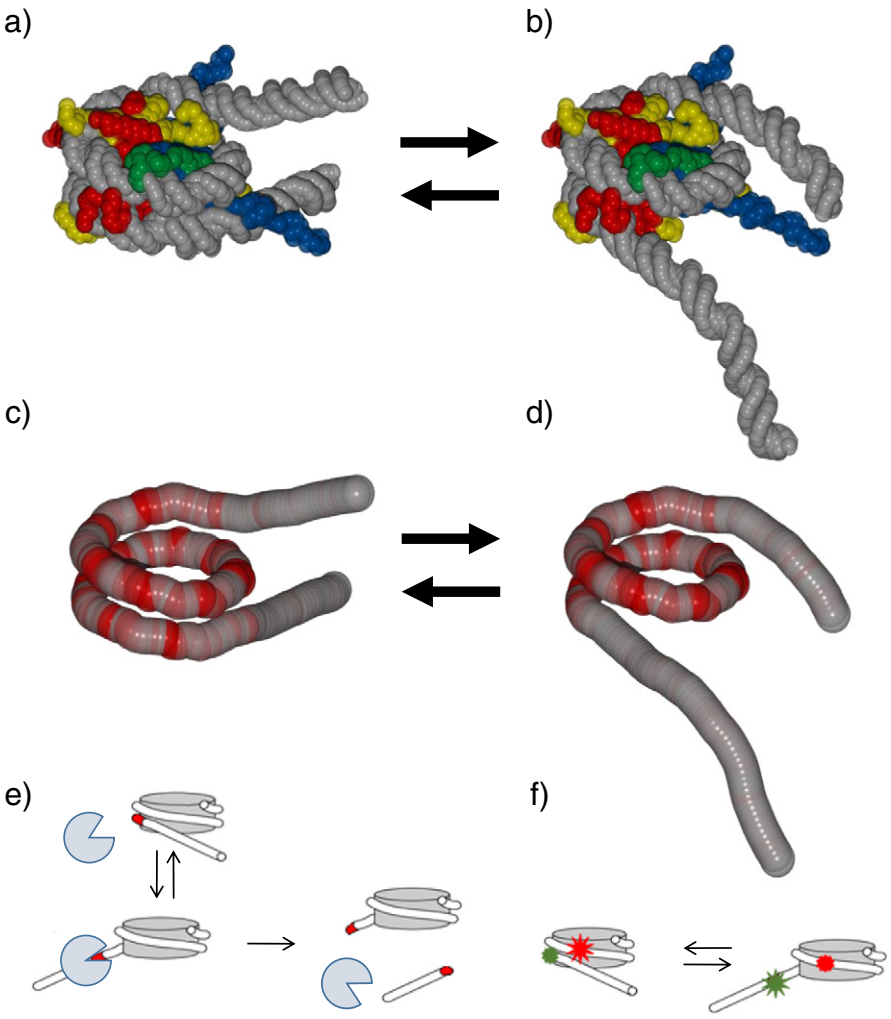

Fig. 2. Nucleosome breathing relieves mechanical stress in the DNA. The energetic penalty for DNA folding into a highly bend nucleosome is balanced by electrostatic interactions between DNA and histones, resulting in a dynamic equilibrium between fully wrapped nucleosomes (a and c) and partially unwrapped nucleosomes (b and d, 30 bp unwrapped). For nucleosomes that do not have favorably distributed dinucleotides, the equilibrium would shift towards the more open structure on the right. Color schemes and coarse graining identical to those of Fig. 1. Nucleosome unwrapping was demonstrated indirectly by enzymatic digestion [40], schematically shown in e) and more directly by single-pair Forster resonance energy transfer [147], schematically drawn in f).

else was changed in the nucleosome: the DNA sequence. This new construct was based on a sequence, called 601, that was extracted from a huge pool of random DNA by selecting for high affinity to nucleosomes [45]. This special non-natural sequence has a higher affinity to nucleosomes than any known natural sequence and has become the most common sequence used for studying nucleosome dynamics.

The questions of histone tail acetylation was then addressed for the 601-nucleosome comparing normal and hyper-acetylated histones extracted from HeLa cells [46]. Again, the effect of tails was modest (a 1.1- to 1.8-fold increase in accessibility). Another experiment [47] modified the same DNA construct to study more directly a well-known sequence effect. This experiment was designed based on the observation that poly(dA) tracts are enriched in promoter regions, suggesting that such sequences provide better access to DNA either by repelling nucleosomes or by having higher equilibrium constants for site exposure. In this experiment, a 16 bp A-tract was incorporated into the 601 sequence either at the end or more towards the middle of the wrapped portion. Independent of the position of the A-tract, the equilibrium constants were found to be lowered roughly 1.6-fold. So here, surprisingly, the effect of sequence was moderate.

A more direct way to follow the breathing dynamics has been achieved with experiments employing fluorescence resonance energy transfer (FRET). In such experiments, a donor and an acceptor dye are attached to the DNA and to the octamer [48,49,50,51,52,53] or both to the DNA molecule [54,55,56,57,58,59,60,61,62,63,64,65,66] (see also a recent review [67]). In the wrapped conformation, the pair of dyes is close in space so that a FRET signal is observed, whereas for the unwrapped configuration the FRET signal is largely absent. A precise measurement of distances is typically not possible due to the rapid decay of the FRET efficiency beyond a certain distance, the Förster radius. In addition, the conformational flexibility of the unwrapped DNA dramatically smears out FRET efficiencies for intermediate distances [68]. A detailed picture can thus only be obtained for sets of experiments with FRET labels at different positions along the wrapped portion, see e.g., [50].

The majority of the FRET experiments has been performed with 601-nucleosomes [48,49,50,51,52,56,60,61,62,63] and confirm the picture that site exposure is the result of the sequential unpeeling of DNA stretches from the ends. There are only a few studies that attempt to measure the effect of sequence on nucleosome breathing and stability. In Ref. [55], three sequences are compared: besides the 5S positioning element, a regulatory sequence from the MMTV promoter and a TATA containing sequence from the yeast Gal10 promoter. The latter two sequences are occupied by nucleosomes in the inactive transcriptional state. All three DNA fragments were labelled by donor and acceptor dyes at sites 80 bp apart. It was found that the 5S sequence features a

15% to 30% higher energy transfer efficiency than the other two sequences. 5S-nucleosomes are also hardly affected by a dilution in nucleosome concentration or an increase in temperature unlike the other two sequences. However, as the authors state, "Physical interpretations of the types of FRET variations detected in these studies can be complicated and somewhat uncertain…" Another study [59] compared FRET signals between 601- and 5S-nucleosomes. Two different FRET pairs were studied, one inside the wrapped portion and one attached to the end of the DNA linkers (the constructs were 170 bp long, the internal labels were at sites 93 bp apart). A substantial shift in FRET populations was observed for the end labeled nucleosomes (but not for the internally labelled pair) when the histones were acetylated. An even larger effect was seen when comparing the two sequences: the 5S-nucleosome showed a much larger conformational heterogeneity than the 601-nucleosome.

Concluding, nucleosome breathing may provide an important pathway for DNA binding proteins to their target sites inside nucleosomes, or "a mechanism for elongation of RNA or DNA polymerase through chromatin" [40] (the ensuing ratcheting mechanism has been studied in detail for RNA polymerase II [69,70]). Experiments show that there is a strong dependence of nucleosome breathing on sequence. An interpretation of these experiments is, however, difficult. Existing computational models [71,72,73,74,75] are only of limited use as they do not yet account for sequence effects. An exception is the computational approach by Chereji and Morozov [76] which has been mainly developed to interpret the high-resolution nucleosome map of *S. cerevisiae* [24] but is also applied to single-nucleosome experiments [40,44]; this approach, however, requires a large number of fit parameters. A visual representation of the effect of sequence on DNA nucleosomal breathing is shown in Fig. 2 C and D.

To come to a clear understanding of nucleosome breathing and how it is affected by sequence, well-designed experiments and computer simulations need to be combined in a common effort.

5. Force-induced nucleosome unwrapping

Soon after the first chromatin fiber stretching experiment [77], mechanical signals from rupture events of individual nucleosomes could be resolved [78]. The first systematic study on force-induced nucleosome unwrapping was presented in 2002 by the Wang lab [79]. The experiment was performed on a DNA template containing 17 5S positioning sequences, the same sequence that was also used in some of the site exposure experiments [40,41,42]. When pulling the system with the help of an optical tweezers, 17 discrete rupture events were observed, corresponding to the unwrapping of the last turn, about 80 bp, of each of the 17 nucleosomes. The mode of unwrapping of the nucleosomal array, sequential, not parallel, and the dependence on the rupture force on the pulling rate hinted at the existence of a kinetic barrier against unwrapping. The authors suggested that the barrier, estimated via force spectroscopy [80] to be about 35 kT, reflects the existence of two strong binding sites, about 40 bp away from the dyad, that stabilize the last DNA turn [79].

The picture of two strong binding sites was challenged by a theoretical study [81], which demonstrated the possibility of a high barrier against unwrapping that results from the nucleosome geometry and DNA elasticity. This model represents the DNA molecule by a homogeneous elastic rod (the wormlike chain model) under an external tension and the histone octamer as a cylinder onto which a section of the DNA is adsorbed. The shape and elasticity of the non-wrapped DNA portion, the arms, can be worked out using Euler's 271-year-old theory of elastic rods. The calculation (detailed in Ref. [82]) shows that the nucleosome – during the unwrapping of the last DNA turn – performs a 180° flip in its orientation (see also [83]). In doing so, the nucleosome crosses an energy barrier with the transition state being the half-flipped nucleosome. The high energy of this transition state comes from two strongly deformed DNA portions in the arms close to the points where the DNA enters the wrapped portion. The surprising finding of this study is that the barrier has not a constant value but is a function of the applied force: the harder one pulls the sharper the DNA needs to bend and the higher the energy. The experiment [79] designed to measure the barrier against unwrapping in fact created the barrier in the measurement. Another insight of this model is that the barrier depends on the DNA stiffness. One should thus expect a strong dependence of the typical (rate-dependent) rupture force on the underlying base pair sequence.

What do experiments tell us about the dependence of the rupture forces on the type of the nucleosome (sequence, histone tail state, mutations…)? There is systematic work on the effect of histone tail modifications [84] and of sin mutations [85] but a systematic study on the role of DNA sequence is dearly missing. Beside the 5S-nucleosome pulling experiments [79,84], there are various experiments with nucleosomes on the 601 sequence, either arranged in arrays [85,86,87,88] or in a single-nucleosome template [89,90,91]. However, differences in the experimental setup, pulling rates, ionic strengths, or histones used make it hard at this point to come to any conclusion concerning differences in the unwrapping behavior of 5S- and 601-nucleosomes. One direct comparison between two experiments ([84] vs. [86]) is presented in Fig. 5 of Ref. [92] and does not show clear dissimilarities despite experimental differences between the two curves. In particular, the pulling rates differ too much to come to any definite conclusion on the role of DNA sequence.

A number of papers presented improved versions of the nucleosome unwrapping model [81]. Electrostatics and hydrodynamics is included in Ref. [93], fluctuations in the spool orientation and DNA arms are accounted for in Ref. [94], repulsion between the two DNA turns in Ref. [95], inhomogeneous strengths of the binding sites in Refs. [96, 97], torque in Refs. [98,99], atomistic details and explicit water in Ref. [100], and its application to DNA-histone H1 toroids in Ref. [101]. The theoretical treatments agree that it is the DNA bending during nucleosome flipping that causes the barrier against unwrapping. Despite this insight and despite the wide range of models employed so far, the theoretical side suffers from the same short-coming as the experimental one: also, here a systematic study of the effect of sequence-dependent elasticity is dearly needed.

One very recent experiment very clearly demonstrates that sequence matters. The Ha lab [91] followed force-induced nucleosome unwrapping in unprecedented detail by combining an optical tweezers setup with FRET measurements. By putting FRET labels at various positions, it was possible to determine which part of the DNA unwraps first when the 601-nucleosome is put under increasing tension. It was found that the nucleosome unwraps asymmetrically. The FRET signal from the pair of dyes close to one end of the wrapped portion decreased substantially below 5 pN, whereas the other end could stand forces in the range of 15 to 20 pN before FRET was lost. The only source of this asymmetry can be the DNA molecule itself (e.g., swapping the direction of the surface tethering had no effect). This was impressively demonstrated by pulling on a nucleosome for which the inner 73 bp of the positioning sequence were swapped: this modified nucleosome unwrapped also asymmetrically, but starting from the other half. The flexibility of the two halves of the wrapped sequence (for the original sequence and the modified one) were determined using a DNA cyclization essay and in both cases it was found that the softer half remains wrapped up to higher forces in the nucleosome pulling experiments.

Summarizing, pulling experiments have taught us that the detailed response of a nucleosome to an external force depends on various internal (DNA sequence, histone tail acetylation, sin mutations) and external (salt concentration) factors. Theoretical efforts have so far investigated various effects (DNA–DNA repulsion, non-uniform histone–DNA interaction) but have not yet accounted for the sequence-dependent DNA elasticity. The above-mentioned recent experiment [91], however, shows that this might be a major effect. It will be important in the future to measure systematically the effect of sequence on nucleosome unwrapping and implement this effect into computational models.

This will allow to discern the relative importance of the various effects on the nucleosome stability under tension and will establish the magnitude of sequence effects.

6. Nucleosome sliding

Nucleosome sliding is a mechanism by which a nucleosome changes its position on a DNA molecule without leaving it in between. The first quantitative experiments under well-controlled conditions were presented by Pennings, Meersseman, and Bradbury [102,103,104]. The authors devised elegant methods to measure nucleosome repositioning using two-dimensional gel electrophoresis. In their first study [102], they showed that on tandem repeats of 5 s rDNA positioning sequences (each of length 207 bp), nucleosomes assemble in one dominant position surrounded by minor positions multiples of 10 bp apart. Most interestingly, there is a dynamic redistribution between these positions. Substantial redistribution took place on a 207 bp DNA fragment when the sample was incubated for 1 h at 37 °C, but not at 4 °C. A set of preferred positions, all multiples of 10 bp (the DNA helical pitch) apart, was observed. In addition, it was found that the nucleosomes have a preference for positioning at the ends of the DNA fragments (see also Ref. [105]), whereas the 5 s rDNA positioning sequence itself was located more towards the middle.

The authors extended their study to head-to-tail dimers of 5 s rDNA (207_2) [103]. Again it was found that when the sample was incubated at elevated temperatures, a repositioning of the nucleosomes takes place. Interestingly, however, the study indicated that the repositioning occurred only within a cluster of positions around each positioning sequence but not between them. This finding indicates that there is no "long-range" repositioning at the low ionic strength used in this study. Other systems were studied in Ref. [103]: fragments of H1-depleted native chromatin and nucleosomes reconstituted on *Alu* repeats. In these cases, a repositioning was also detected as a result of an elevated temperature incubation, but results were not quantitative enough to make definite comparisons to the 5S positioning element. The authors concluded that the repositioning "may be visualized as following a corkscrew movement within the superhelical path of the DNA" [103]. In another paper [104] the authors measured nucleosome mobility on the 207_2 dimer in the presence of linker histone H1 (or its avian counterpart H5) and found that it was dramatically reduced.

Ura et al. [106,107], following [103], studied nucleosome mobility on the 207_2 dimer under varying conditions, namely, in the presence of various chromosomal proteins and in the case when the core histones were acetylated. In the former case, mobility was suppressed (depending on the type and concentration of the chromosomal protein); in the latter case, there was no significant change in the mobility. That tails can influence nucleosome mobility nevertheless was demonstrated by Hamiche et al. [108]. They found that the nucleosome mobility along DNA depends on the presence of histone tails. In particular, in the absence of the N-tail of H2B that passes in between the two turns of the nucleosomal DNA [4], spontaneous repositioning of the nucleosomes was detected.

Flaus et al. [109] developed a different strategy to follow nucleosome positioning and repositioning with bp resolution using chemically modified H4 histones that induce, after addition of hydroxyl radicals, a strand cleavage close to the nucleosomal dyad (as mentioned earlier, a method now also applied for the in vivo nucleosome mappings [24,25]). Using this method, Flaus and Richmond [110] studied the nucleosome dynamics on an MMTV sequence, which revealed several features of repositioning more clearly. The longest fragment, 438 bp, of this sequence had two positioning sequences where two nucleosomes assembled, each at a unique position. These positions were also found when mononucleosomes were assembled on shorter fragments that included only one of the two positioning sequences. The authors determined the degree of repositioning of the mononucleosomes on such shorter fragments (nucleosome A on a 242 bp fragment and nucleosome B on a 219 bp fragment) as a function of heating time and temperature. It was found that the repositioning rates increase strongly with temperature but also depend on the positioning sequence and length of the fragment. The difference in repositioning for the two sequences is remarkable: at 37 °C, one has to wait <90 min for the A242 and more than 30 h for the B219 substrate to have half of the material repositioned. For the slower B-nucleosome, the set of new positions were all multiples of 10 bp apart whereas the more mobile A-nucleosome did not show such a clear preference for rotational positioning. The authors argued that these differences reflect specific features of the underlying bp sequences. Nucleosome B is complexed with a DNA sequence that has 10 bp periodic AA/AT/TA/TT dinucleotides, whereas nucleosome A is positioned via homonucleotide tracts. Of course, any DNA sequence will have specific distributions of these dinucleotides that may dictate specific preferred sites for (re-)positioning of nucleosomes, Fig. 3.

Of interest is also an experimental approach by Gottesfeld et al. [111]. The authors studied repositioning on a 216 bp DNA fragment that contained the 5S rDNA positioning sequence but this time in the presence of pyrrole-imidazole polyamides, synthetic minor-groove binding DNA ligands that are designed to bind to specific target sequences. Experiments were performed in the presence of one of four different ligands, each of which had one binding site on the wrapped DNA portion. It was found that a 1-h incubation at 37 °C in the absence of any ligand leads to redistribution of the nucleosomes. Remarkably, this redistribution was completely suppressed in the presence of 100 nM ligands if (and only if) the target sequence of this specific ligand faces outside (towards the solution) when the nucleosome is at its preferred location along the DNA.

Finally, we mention an experiment by Flaus et al. [112] where it was found that mutations in histone proteins can have a profound impact on nucleosome mobility. Nucleosomes containing histones that feature a sin mutation (which weakens the strong binding sites close to the dyad) show about 4 times faster sliding.

Most of the above-mentioned experiments worked, for experimental reasons, with DNA containing nucleosome positioning sequences. This is in sharp contrast to work on nucleosome sliding on telomeric DNA [113,114,115,116,117]. Telomeric DNA sequences feature short repeat sequences, typically 6–8 bp in length, that are not commensurate with the DNA helical pitch and can therefore not contain the strong 10 bp undulations typical for nucleosome positioning sequences. It was found that nucleosomes reposition substantially faster on telomeric DNA than on average DNA [116].

What is the physical mechanism behind nucleosome sliding? In a series of papers one of us (HS), in collaboration with others, calculated possible scenarios [118,119,120,121,122,123,124,125]. A "sliding" motion in the ordinary sense, i.e., a rigid body-like motion of the DNA around the octamer, is far too expensive as it would require that all binding sites break at the same time; this would cost about 75 k_BT [82,126]. A rolling motion of the octamer, breaking sites at one end and closing sites on the other, is also not possible because a fully wrapped nucleosome has no sites to roll on. This suggest that the mobility of nucleosomes is caused by spontaneously formed small defects in the wrapped DNA portion. Two possible defects are bulged loops and twist defects [82,126,127,128,129,130]. The formation of a loop starts by the spontaneous partial uncoiling from one end (as discussed in the section on nucleosome breathing). In a second step, the partially uncoiled DNA is recaptured starting from a point that is displaced along the DNA such that a bulged loop forms. In a third step, this loop diffuses around the nucleosome before it falls off at either end. If and only if it leaves the nucleosome at the end opposite to the one where it had been created, it causes a net translocation step of the nucleosome along the DNA by an amount that corresponds to the extra length that had been stored in the loop. The second mechanism consists of twist defects that can also form at either end and diffuse to the other end. A twist defect contains either a missing or an extra base pair. To accommodate this defect, DNA needs

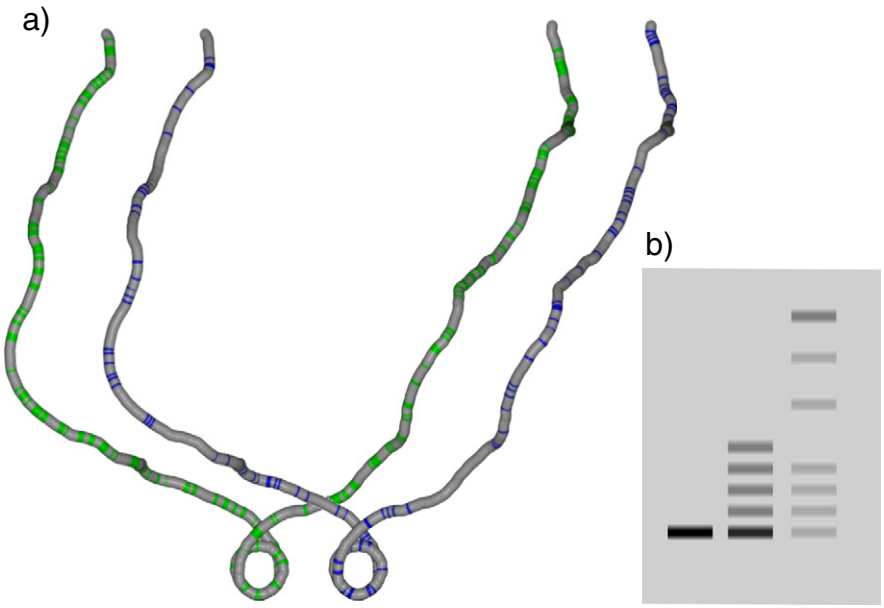

Fig. 3. In a 1000 bp random DNA sequence, the statistical distribution of AT, AA, TT, and GC dinucleotides yield multiple locations where a nucleosome can be positioned to optimally make use of these dinucleotides. Repositioning of the nucleosome, be it spontaneous or with the help of chromatin remodelers, will modify the overall stability, as well as nucleosome breathing, according to the local sequence imposed energy landscape. Color schemes and coarse graining identical to those of Fig. 1. b) The distributions of nucleosome positions after thermal or enzymatic remodeling can be resolved by native gel analysis [168], as schematically depicted in b) and typically yield pronounced preferences for nucleosome positions that could be related to DNA sequence.

to be either overtwisted and stretched or undertwisted and compressed. A twist defect that diffuses from one end to the other end in a nucleosome causes its translocation by one base pair.

Which type of defect causes the translocation of the nucleosome along DNA in the experiments? The energy cost to form a loop has two contributions, desorption and bending, and has been estimated to be about 20 k_BT for the case of a 10 bp excess length (the cheapest small loop) [118,119] (see also [131]). This has to be compared to twist defects which are energetically much cheaper, about 10 k_BT for an extra or missing base pair [120]. The predicted mobilities differ vastly for the two types of defects: the typical redistribution times of a nucleosome on a 200 bp fragment are on the order of hours for the loop mechanism and of seconds for twist defects [126]. In addition, would loops shift nucleosomes to new positions, these positions would be 10 bp (the length transported by the cheapest small loops) apart from each other [119], whereas twist defects shift nucleosomes by one bp steps. It seems that loop defects are consistent with experiments such as presented in [102] but not with twist defects. The problem, however, is that loops should play a negligible role since twist defects are much more common and should lead to much more mobile nucleosomes than observed in experiments.

The solution of this riddle lies in the sequence-dependent DNA elasticity that has not been accounted for in the above discussion. Experiments are typically performed on strong positioning sequences, starting with a nucleosome in an energetically preferred position. If the nucleosome moves now to the right by 10 bp, one helical pitch, due to twist defects, the DNA needs to perform one full turn of a corkscrew movement, and thereby it has to cross a high barrier that is located about 5 bp to the right from the starting position. This barrier slows the motion down substantially. On the other hand, the loop-mediated translocation moves in steps of 10 bp and thereby circumvents such barriers.

To better understand the detailed mechanics underlying nucleosome mobility, a computational model was developed that accounts for the sequence-dependent DNA elasticity [125]. In this approach, the DNA is represented by the rigid base pair model, which is forced into the conformation that corresponds to DNA inside the nucleosome crystal structure [4], and the strength of individual binding sites is estimated from Ref. [13]. It was found that the highest barriers to cross for a twist defect wandering through a nucleosome are close to the dyad where the strongest binding sites need to be broken. Moreover, a 13% reduction of the strength of these sites can account for the 4 times higher mobility seen in the experiment with sin mutant nucleosomes [112]. Most importantly, the model predicts that a nucleosome sliding along the 5S positioning element has to cross barriers of about 10 k_BT height every 10 bp (if the nucleosome repositioning is caused by twist defects). The surprising conclusion is that – on theoretical grounds – repositioning rates are expected for both mechanisms, loops and twist defects, to be comparable when one accounts for sequence-dependent DNA elasticity. Also, the 10 bp spacing of observed nucleosome positions is consistent with both scenarios.

The theoretical models are not precise enough to determine which of the two scenarios is dominating. It is even possible that both mechanism would be at work at the same time and cause the experimentally observed nucleosome sliding. However, two experiments are in favor of twist defects as the cause of nucleosome mobility. One is the above-mentioned experiment by Gottesfeld et al. [111], where it was found that nucleosome mobility is suppressed in the presence of minor-groove binding ligands. A quantitative theoretical analysis [121,123] showed that this finding is consistent with the twist defect picture whereas it is hard to see why the ligands would suppress bulged loops should they be present. Another observation in favor of twist defects is the increased mobility found on telomeric DNA [113,114,115,116,117], where the elastic energy landscape shows much smaller undulations with a periodicity set by the telomeric repeat length [125]. Nucleosome repositioning is thus yet another nucleosome property that depends dramatically on the mechanical properties of the involved DNA. Nucleosomes that are pinned on strong positioning elements like 601 might not move at all even if other quite deep local minima are close by whereas nucleosomes on telomeric DNA are intrinsically highly mobile.

We conclude this section by mentioning that DNA loops on nucleosomes might play an important role in the elongation of RNA polymerases through nucleosomes. After a polymerase has entered deep enough into a nucleosome (through the ratcheting mechanism mentioned earlier), it might be caught into a loop that travels with the RNA polymerase around the nucleosome. As a result, the polymerase does not only

get around the nucleosome but the nucleosome is moved upstream by the amount transported inside the DNA loop [69,132,133,134]. The details of this mechanism might be more involved but a looped intermediate seems typically play crucial role [135,136,137]. Remarkably, it might be even serve as a sensor for DNA damage [138].

7. Dissociation/partial assembly

So far, we have only considered full nucleosomes composed of 147 bp of DNA and two of each of the core histone proteins. This, however, is an oversimplification of the many shapes a nucleosome can assume [139].

In the assembly of nucleosomes, both *in vivo* [140] and *in vitro* [141] nucleosomes form in a modular fashion. *In vitro* reconstitution is typically achieved by salt dialysis, reducing the salt concentration slowly from 1 M to 100 mM, during which first a tetramer consisting of $(H3–H4)_2$ wraps 80 bp of DNA, followed by the association of two H2A–H2B dimers. On short DNA substrates, it is essential that the tetramer positions right in the middle in order to leave sufficient DNA on both sides for the dimers. In this perspective, the central 80 bp of DNA plays a crucial role, and it is not surprising that the characteristic 10 bp periodic dinucleotide signals found in good positioning sequences are most prominent in the central regions of the nucleosome [11].

It is well known that incorrect ratios of histones and DNA, as well as too fast reconstitution, can generate subassemblies. A host of alternative structures involving histones and DNA has been reported, ranging from tetrasomes, hexasomes to altosomes [142]. Engeholm et al. report that assemblies of a hexasome and a nucleosome can form a single particle containing three wraps of DNA [143]. Such structures were not only obtained after reconstitution on truncated 601 dimers but could also be observed after heat-shift and enzymatic remodeling reactions on MMTV-based substrates, indicating a relation between partial (dis-)assembly, nucleosome sliding, and alternative histone–DNA structures. Interestingly, the abundance of these alternative structures may not only depend on buffer conditions, posttranslational modifications [142], and histone variants, but also DNA sequence.

Both *in vitro* and *in vivo*, such partial or aggregated histone–DNA assemblies can be converted into canonical nucleosomes by chaperones like ACF [144]. In fact, converting non-nucleosomal aggregates into canonical nucleosomes may be a major function. Moreover, the tetrasome or other subassemblies may be genuine intermediates during remodeling reactions, incorporation of histone variants, and transmission of epigenetic patterns during replication [139]. It is difficult to assess the role and abundance of alternative nucleosome structures *in vivo* because of their transient nature. In nucleosome mapping studies, a significant fraction of the reads are shorter or longer than 147 bp, but that could also originate from the preparation procedures. It is clear though from FRAP experiments that H2A–H2B dimers are much more mobile than their H3H4 counterparts [145].

One reason for the higher mobility of H2A–H2B dimers *in vivo* may be that transcription has been associated with the loss of a dimers. In an elegant *in vitro* experiment, Kireeva et al. demonstrated that “as Pol II progresses along a gene, it transiently converts nucleosomes on its way to hexasomes” [146]. Given the consequences of this partial dissociation of the nucleosome for DNA accessibility *in vivo*, nucleosome positioning and chromatin structure remain to be resolved.

In single-molecule experiments, chromatin samples are generally diluted down to sub-nanomolar concentrations, which challenges the stability of the nucleosomes, although in some cases, additional nucleosomes can be added to the sample to maintain μM concentrations and nucleosome integrity. Moreover, the close proximity of surfaces is generally detrimental to the nucleosome [147], so is prolonged exposure to forces during force spectroscopy experiments [88]. This nucleosome fragility puts high demands on the experiments and may perhaps explain the strong focus on the unwrapping of the last 80 bp in many of the force spectroscopy studies. In FRET studies, there may be significant fractions of nucleosomes that have partially dissociated and these should be explicitly excluded from the analysis to get an objective measure of the nucleosome, for example, by proper placements of fluorescent labels and by alternating excitation (ALEX) [67].

An emerging question is how much of the assembly and disassembly behavior is affected by DNA sequence. Almost all detailed *in vitro* studies have been performed with selected nucleosome positioning sequences, the vast majority with the Widom 601 sequence, and it seems likely that this sequence is not representative for natural DNA. With the H2AH2B dimers as the most dynamic parts of the nucleosome, it is likely that they will feature a lower affinity for non-601 DNA. In this perspective, it will be essential to compare various variations of these sequences.

8. Nucleosome–nucleosome interactions and linker DNA

Nucleosomes in dense solutions tend to stack, mediated by the H4 tails, as demonstrated in crystal structures and recent cryo-EM [148]. In strings of nucleosomes, such stacking can drive the formation of higher-order structures like the highly debated 30 nm fiber. Chromatin fibers containing 20 bp linker DNA fold *in vitro* into a two-start fiber in which the DNA zigzags from one nucleosome to the next [149,150]. Such stacking of nucleosomes in two-start helices maintains relatively straight linker DNA, although the crystal structure of the tetranucleosome shows significant local deformations in the linker DNA, Fig. 4A and B. Recent modeling reveals tight constraints on the linker DNA in terms of twist [151]. FRET experiments on tri-nucleosomes feature several time constants for conformational dynamics in these fibers [152].

Systematic studies on fibers with larger linker lengths report more dispersed results. Robinson et al. show that increasing the linker length between 30 and 60 bp does not affect the diameter of the fiber, arguing for a solenoid folding in these condensed fibers [8]. Other EM results hint at a more open structure, with straight linker DNA and little direct contact between nucleosomes, arguing for a disordered fiber. Cross-linking studies by Richmond et al. report two-start topologies for 20 and 25 bp linker DNA [149]. Using force spectroscopy on folded fibers with 50 bp linker DNA, Kruithof et al. found that the force-extension curves at low force can best be interpreted in terms of a one start helix, based on the maximal extension before rupture, the small stiffness compared to 20 bp linker DNA fibers, and the non-cooperativity of the rupturing of stacked nucleosomes [153]. In this case, the linker DNA should be strongly bent. Recently, a more detailed analysis of the force-extension experiments suggested that some of the nucleosomal DNA is unwrapped in folded chromatin fibers, which would partially relieve the stress on the linker DNA [88].

In FRET studies on nucleosome breathing in di-nucleosomes, 50 and 55 bp apart, it was clear that the presence of a neighboring nucleosome results in more open nucleosomes, with the 55 bp linker DNA being more effective in stimulating its neighbor's breathing than the 50 bp linker [154]. Given the ease with which the nucleosome can unwrap some of its DNA, it is highly likely that interacting nucleosomes, with 50 bp of linker DNA in between, partially unwrap to release some of the bending stress, as shown in Fig. 4C and D. Consistent with this, enzymatic digestion experiments by Poirier et al. show that incorporation of a nucleosome in a folded fiber can increase DNA accessibility up to 8-fold, pointing to a perhaps dynamically disordered structure of the chromatin fiber [155]. It should be noted that, despite strong efforts, it has not been possible to resolve clearly structured 30 nm fiber in chromatin in cells [156], questioning the existence of regular fibers *in vivo*.

The length of linker DNA found *in vivo* varies between 10 and 70 bp, i.e., a fraction of the bend and twist persistence length of DNA. Even when some of the nucleosomal DNA is added, this short length either constrains the conformational freedom of two neighboring nucleosomes, or can put high stress on the linker DNA when nucleosomes interact. Like in any case of mechanical stress on DNA,

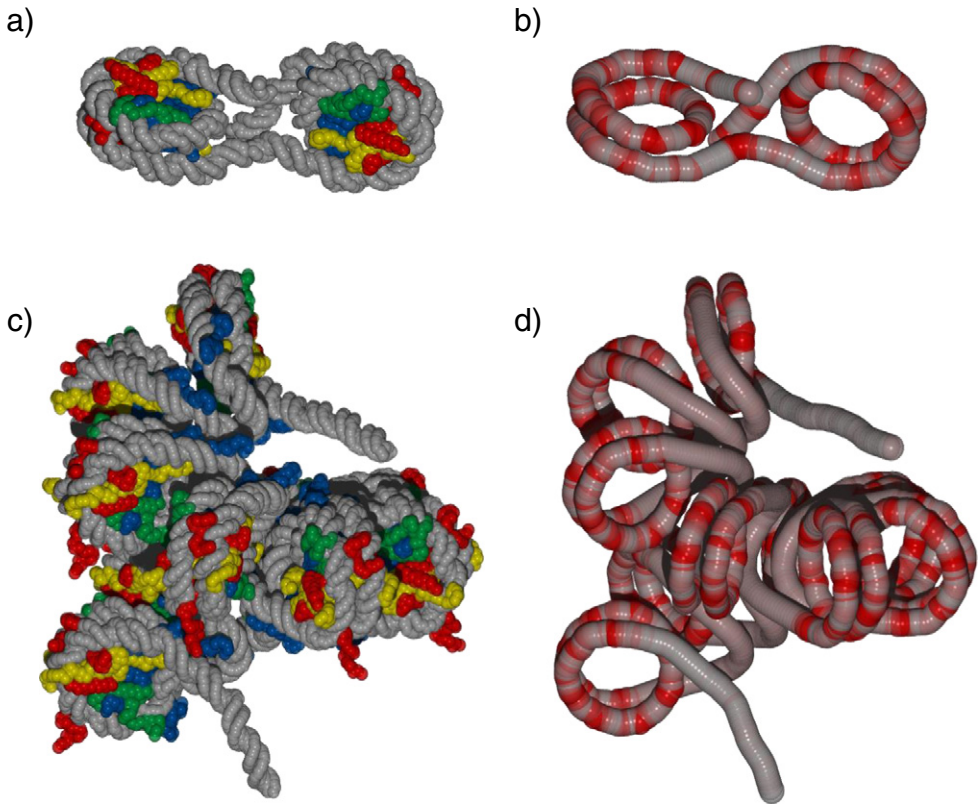

Fig. 4. Higher-order folding of nucleosomal arrays into chromatin fibers leads to strong compaction of DNA and puts mechanical constraints on the linker DNA. (A and B) Half of the structure of a tetranucleosome 1ZBB [150] that was proposed to represent chromatin folding in fibers with 20 bp linker DNA. Note that the deformation of the linker DNA locally has a similar magnitude as in the nucleosomal DNA. (C and D) A tentative model of a left-handed chromatin fiber containing 10 nucleosomes and 50 bp linker DNA. The linker DNA was modeled by connecting nucleosomes distributed in a 33 nm super helix, while constraining rise, roll, and twist along the DNA path. Unwrapping of 20 base pairs of DNA on both sides of the nucleosome was allowed (details to be published elsewhere). The large bending of the linker DNA, as well as steric constraints, has been argued to preclude folding into a solenoidal structure. A 3D visualization of the path of the DNA and its deformation in this model shows that these can be overcome. Although the linker DNA in this model does not have as large deformations as the nucleosomal DNA, it's slightly pink color indicates that the DNA is significantly bent. The distribution of specific dinucleotides along the linker DNA may favor or disfavor a specific higher-order folding. Color schemes and coarse graining identical to those of Fig. 1.

the sequence of the linker DNA may play an important role relieving some of this stress, when the linker DNA happens to consist of a sequence that favors bending or twisting in a certain direction. We speculate that there may be sequence rules for linker DNA that define or modulate higher-order folding, not unlike the rules that govern nucleosome positioning. Unfortunately, we are not aware of any (experimental) study that systematically tests the effect of linker DNA sequence on chromatin folding. Whereas the nucleosomal DNA is generally well defined, the linker DNA sequence is usually not considered, which may have biased the outcomes of chromatin folding studies.

Unlike the highly regular chromatin fibers used *in vitro*, native chromatin features highly variable linker lengths and sequences. Thus, each pair of nucleosomes is unique and will have its own likelihood to stack on each other, or refrain from interactions. The consequences for chromatin folding in dense fibers may need to be evaluated nucleosome by nucleosome. To understand the role of chromatin folding on transcription factor binding, it will thus be essential to take into account the entire local environment in a 3-dimensional fashion rather than a simplistic linear arrangement of nucleosomes and other factors on straight DNA. Although this may seem an endless endeavor, when the recent progress on the physical properties of DNA as a function of DNA sequence (see e.g., [157]) is put to use in a chromatin context, we may be able to extract sequence rules for chromatin folding, like those that are developing for nucleosome positioning.

9. Theoretical approaches to deal with DNA sequence in nucleosomes

Most nucleosome models have typically neglected sequence effects and have modelled the DNA as a homogeneous elastic rod [81,93,94,95,96,98,99,118,119,158]. Also, the interaction between the DNA and the histone octamer has typically been modelled to be continuous. The level of details that is now experimentally available makes it necessary to create more detailed models that account for the sequence-dependent elastic properties of DNA. Due to the large size of the nucleosome complex one typically needs to use a coarse-grained description of the nucleosome, especially of the nucleosomal DNA. There exist now such models, several based on the rigid base pair model where the DNA conformation is described by the positions and orientations of its base pairs that are modelled as rigid plates [159,160,161,162]. A less coarse-grained description, the rigid base model, which allows also for the six degrees of freedom between the bases of a pair [157,163], is a new very promising alternative for the future.

Several nucleosome models have already been presented that account for the sequence-dependent elastic properties of DNA. Most of these models are based on variants of the rigid base pair model. Tolstorukov et al. [19], Vaillant et al. [164], and Morozov et al. [165] focused on nucleosome positioning whereas Becker and Everaers [166] worked out the forces and torques on individual bases in the nucleosome. Fathizadeh et al. [125] focused on nucleosome sliding as discussed already in a previous section. In general, these models

are quite successful in predicting nucleosome positioning, especially when one allows for the relaxation of the DNA molecule into its sequence-dependent conformation [165].

What is missing so far is, however, the application of these models to the wide range of experiments discussed in this review. Especially models that account for the unpeeling of DNA, spontaneous or forced, have not yet been developed and should give deeper insight about how good these models really are in predicting details in the unwrapping energetics and dynamics. Likewise, sequence-dependent DNA models can be used to study the elastic costs of linker DNA bending inside various chromatin fiber geometries. Finally, such models will allow design of DNA molecules with special properties, e.g., of DNA stretches with large nucleosome affinities, of nucleosomal sequences that guide the force-induced unwrapping along pre-described paths or of linker DNA sequences that induce fiber folding into certain geometries. We are currently implementing these possibilities.

10. Conclusion

This review on the nucleosome has focused on a feature of this DNA-protein complex that in our opinion has not yet gained enough appreciation: the dependence of its physical properties on the base pair sequence that is wrapped into the complex. There are striking but still rather non-systematic observations on the massive influence of DNA sequence on the static and dynamical properties of nucleosomes. We have given some examples in this review: the positioning of nucleosomes is strongly affected by DNA sequence and their sliding along the DNA molecules is dramatically reduced when a nucleosome sits on or nearby a nucleosome positioning sequence. The accessibility of wrapped DNA through spontaneous unwrapping, the site exposure or breathing mechanism, is dramatically modulated by the involved sequence. Likewise the response of nucleosomes to tension is largely determined by the sequence, e.g., a non-symmetric (i.e., non-palindromic) nucleosomal sequence causes typically the asymmetric unspooling of such a nucleosome under tension. The assembly and disassembly behavior of a nucleosome is also affected by DNA sequence, which is one of the reasons that so far experiments have focused on very few and, presumably, not representative sequences. The role of DNA linkers between nucleosomes for guiding the folding of the higher-order structure has also so far not been studied but may be of crucial importance as well. We believe that it is now time to address these questions systematically in a combined experimental and theoretical effort.

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