The nucleosome: from structure to function through physics

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Abstract

Eukaryotic cells must fit meters of DNA into micron-sized cell nuclei and, at the same time, control and modulate the access to the genetic material. The necessary amount of DNA compaction is achieved via multiple levels of structural organization, the first being the nucleosome – a unique complex of histone proteins with $\sim$ 150 base pairs of DNA. Here we use specific examples to demonstrate that many aspects of the structure and function of nucleosomes can be understood using principles of basic physics, physics-based tools and models. For instance, the stability of single nucleosomes and the accessibility to their DNA depends sensitively on the charges of the histones that in turn can be changed by post-translational modifications. The positions of nucleosomes along DNA molecules depend on the sequence-dependent shape and elasticity of the DNA double helix that has to be wrapped into the nucleosome complex. Larger-scale structures composed of multiple nucleosomes, *i.e.* nucleosome arrays, depend in turn on the interactions between its constituents that result from delicately tuned electrostatics.

Keywords: epigenetics, chromatin structure, partially assembled nucleosome structures, nucleosome positioning, post-translational modifications, nucleosome arrays

1. Introduction

The important role of chromatin structure in key cellular processes such as cell differentiation, DNA replication, repair, transcription, and epigenetic inheritance, *i.e.,* inheritance that is not coded by the DNA sequence, is now well recognized [1], Fig. 1.

Uncovering relationships between molecular structure and biological function is never easy. While sometimes the biological function can be related to structure in a relatively direct way, as in the case of some enzymes with well defined active sites and mechanism of action, the relationship can also be very complex, involving *e.g.* subtle dynamics of

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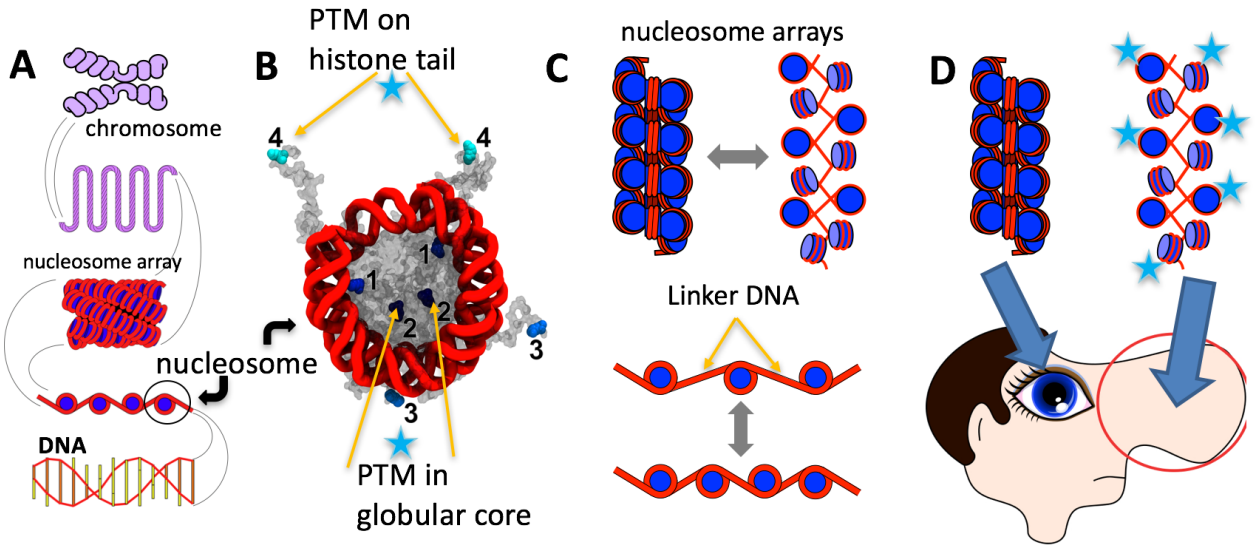

Figure 1: (A) Compaction of the DNA (chromatin) in eukaryotic cells is a complex hierarchy of various structures controlled by multiple modulating factors. (B) The structure of the primary level of the DNA compaction – the nucleosome – is relatively well-defined. Various post-translational modifications (PTM), such as acetylation of lysine residues, modulate the state of the nucleosome, including accessibility of its DNA. Shown are 4 examples of lysine acetylation sites, 1-4: H3K56, H4K91, H2BK5, H3K4. Positively charged N-terminal histone tails facilitate the condensation of the net negatively charged nucleosomes into arrays. (C) Nucleosome arrays are likely represented by a variety of structural forms, depending on the subtle interplay between several modulation factors. The arrays might switch between structures with different levels of compaction (top) or the nucleosomes might occupy different sets of positions (bottom). (D) The state of chromatin affects vital processes such as gene expression and cell differentiation; cell types (e.g. eye vs. nose) can be different even though their DNA is identical. Deciphering this structure-function connection in chromatin remains a fundamental unsolved problem in modern biology.

the macromolecule. However, compared to traditional structural biology, which studies relationships between macromolecules, such as proteins and nucleic acids, and their biological function, making connections between chromatin structure and its function is expected to be much harder. The reasons for the difficulty are many. Compared to proteins, the degree of compaction that the DNA undergoes as it “folds" into the cell nucleus is enormous [2]: depending on the organism, about one meter of the DNA must fit within the space of only several microns across. Eukaryotic cells achieve the necessary amount of DNA compaction via multiple levels of structural organization, many of which are still poorly understood. Structures and functions of these chromatin components can be modulated by a myriad of factors *in-vitro* and *in-vivo*. And while the structure of *e.g.* myoglobin is the same in all cell types of the same organism, that may not be true of chromatin structure [3].

The good news is that, despite the inherent complexity, certain basic principles and physics-based methods still operate at all levels of biological complexity – these principles and methods help guide reasoning, explain experiments, and generate testable hypotheses. For example, classical electrostatics, thermodynamics, and physics-based simulations

proved extremely fruitful in traditional structural biology. Here we use several examples to demonstrate that many of the same basic physical principles, physics-based techniques and reasoning can be just as useful in deciphering structure–function connections in the nucleosome.

It is the opinion of the authors that despite the seemingly daunting complexity of the relevant structures and structure-function connections, physics-based approaches can be very useful in the field of epigenetics and chromatin. The review is aimed to support this opinion with examples, rather than to provide a comprehensive account of the field.

2. The nucleosome

The primary level of the DNA packaging in eukaryotic organisms is the nucleosome [4–6], Fig. 1. The structure [7] of the nucleosome core particle, to which we refer to as the nucleosome for simplicity, consists of 147 base pairs of DNA tightly wrapped ≈ 1.75 superhelical turns around a roughly cylindrical protein core. The core is an octamer made of two copies of each of the four histone proteins H2A, H2B, H3, H4. Chromatin compaction at the nucleosome level (and also the next level of nucleosome arrays, discussed further in this review), is believed to be the most relevant to gene access and recognition [8].

2.1. Connection to function through DNA accessibility

Increases in nucleosomal DNA accessibility as small as 1.5-fold can have significant biological consequences, *e.g.* up to an order of magnitude increase in steady-state transcript levels [9] and promoter activity [10]; importantly, these biological consequences of increased DNA accessibility are not sequence-specific, *i.e.* the effects appear to be the function of the increased DNA accessibility *per se*. Thus, studying the DNA accessibility in the nucleosome, and how it can be controlled, is of critical importance for establishing structure-function connections at this primary level of chromatin compaction. Note that the very term "accessibility" may have different meanings depending on the context, *e.g.* "solvent accessibility" of a DNA base means that it can make a steric contact with a nearby solvent (water) molecule. For chromatin compaction at the nucleosome level, one possible functionally relevant definition of DNA accessibility is that the DNA fragment is accessible if it is far enough from the histones so that a typical nuclear factor such as PCNA can fit onto the DNA; in quantitative terms that means at least ~ 15 Å distance from the nearest histone atom [11]. By this definition, all of the DNA in the X-ray structure of the nucleosome [7] is inaccessible to protein complexes that perform, or initiate, transcription, recombination, replication, and DNA repair. However, structural fluctuations can make fragments of the DNA spontaneously accessible. A strong argument can be made [12] in favor of the important role of spontaneous DNA accessibility in gene regulation, despite the ubiquitous activity of ATP-dependent remodeling enzymes that can use energy to expose DNA target sites.

2.2. How stable is the nucleosome?

Spontaneous accessibility of nucleosomal DNA is directly related to the strength of its association with the histone core [13], so the first question one asks is how strong that

association is at physiological conditions, that is how stable is the nucleosome? As it turns out, the question itself, and available answers to it, are not as simple and unique as one may wish them to be. By analogy with protein folding or protein-ligand binding, seemingly the most straightforward measure of the nucleosome stability is the negative of the free energy ΔG^u required to completely unwrap and remove the DNA off the intact histone octamer. However, that quantity has not been directly accessible in experiment [14, 15]. Nevertheless, estimates of upper and lower bounds on $|\Delta G^u|$ can be deduced from available experimental estimates of other related quantities. For example, an upper bound on $|\Delta G^u|$ of 34 kcal/mol can be inferred [16] from single-molecule experiments [17] in which the DNA was gradually (but not fully reversibly) pulled off the histone core. A simple electrostatic model [18] explains the "all-or-nothing" nature of the unwrapping of the last turn of the nucleosomal DNA observed in that experiment. A much higher upper bound of $|\Delta G^u| \sim 150$ kcal/mol was also reported, based on the salt dependence of oligocation-DNA binding [19]. A lower bound on $|\Delta G^u|$, 23 kcal/mol, can be deduced [16] from estimates of the DNA to histone core contact energy obtained [2] from equilibrium DNA accessibility measurements [20]. A theoretical estimate [16] of $\Delta G = -38 \pm 7$ kcal/mol at physiological conditions and relevant nucleosome concentration in the nucleus falls within the above upper and lower bounds. The strong affinity of the nucleosomal DNA to the histone octamer is a consequence of the electrostatic pull between the large and opposite charges of the globular histone core and the DNA, Fig. 2 (A), amplified by the low dielectric environment of the complex. Note that we are tacitly assuming the implicit solvent framework [21] in the discussion of the role of electrostatics in the nucleosome stability. Within this framework, all of the solvent effects, including entropic contributions of the water and mobile ions, are absorbed into the effective free energy. An alternative picture of the DNA-histone binding process that considers explicit contributions of counter-ions can be found elsewhere [22, 23]. We believe that the two pictures are complimentary, but can not pursue a more detailed discussion in this short review. While the above estimates of ΔG^u span quite a range, they all point to one important conclusion: the likelihood, $\exp(\Delta G^u/k_BT)$, that all of the nucleosomal DNA spontaneously unwraps off the unmodified histone octamer under physiological conditions is zero for all practical purposes. Thus, the nucleosome complex *as a whole* is extremely stable [16, 24], much more so than typical proteins (folding free energy is a few kcal/mol), where marginal stability is believed to be beneficial to function.

The extremely high stability of the nucleosome as a whole is clearly conducive of its function as the “information vault" that protects the DNA, but that same high stability presents a challenge to understanding exactly how the cell exercises controlled, on-demand access to the DNA of the various cellular machinery responsible for key processes such as transcription. For example, exactly how RNA polymerase machinery gains access to the nucleosomal DNA remains a fundamental open question in biology [25, 26].

2.3. Access to nucleosomal DNA is facilitated in several ways

A number of studies that characterize the thermodynamics and kinetics of the histone-histone and histone-DNA association in the nucleosome have provided important clues. The emerging picture is that despite its high thermodynamic stability as a whole, the nucleo-

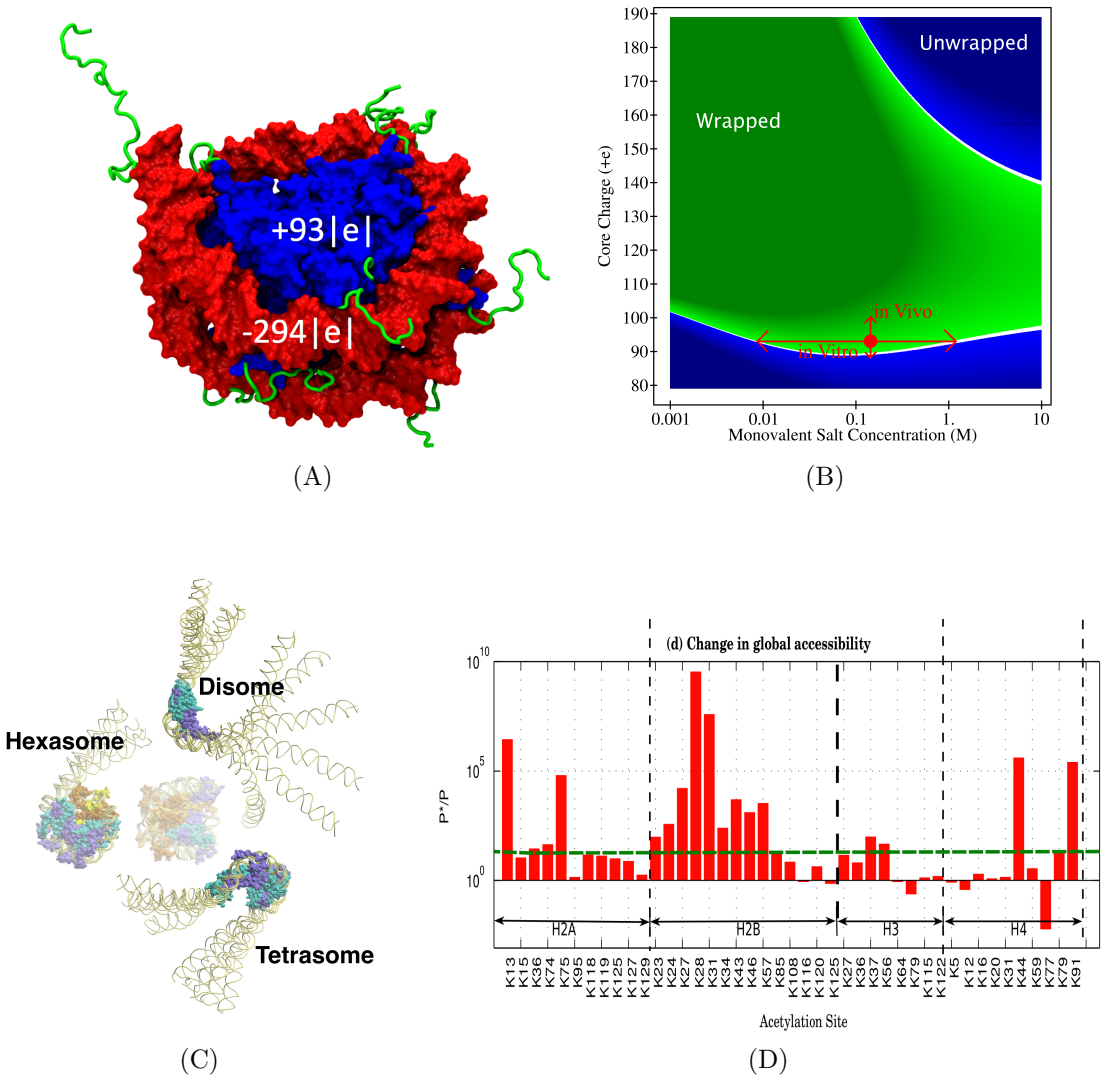

Figure 2: While the nucleosome as a whole is highly stable, access to its DNA can be facilitated in a number of ways. (A) The high stability of the nucleosome stems mainly from the strong electrostatic attraction between the oppositely charged globular histone core (blue) and the DNA (red) [16, 27]. Contribution of the histone tails (green) to the over-all stability of the nucleosome is relatively small [28]; the tails affect partial unwrapping of the DNA ends [29] and may have an effect on the nucleosome core structure [30]. (B) At physiological conditions, the state of the nucleosome (red dot) is close to the phase boundary separating it from the "unwrapped" states where the DNA is more accessible – a small drop in the charge of the globular histone core can significantly lower nucleosome stability, and thus increase DNA accessibility [16]. (C) Conformational ensembles of partially assembled nucleosome structures (PANS) [11]: hexasome, (H2A·H2B)·(H3·H4)$_2$ · DNA; tetrasome, (H3·H4)$_2$ · DNA; and disome, (H3·H4) · DNA. Significant portions of the DNA become accessible in PANS as a consequence of partial histone removal from the nucleosome (2(H2A·H2B) · (H3·H4)$_2$ · DNA.) (D) Effect of all possible lysine acetylations in the globular histone core on the DNA accessibility: while most are predicted to increase the accessibility, few (*e.g.* $H4K77^{Ac}$) may have the opposite effect [31].

some is not a single static structure, but rather a highly dynamic family of interconverting structural states [15, 32–37], in some of which the DNA accessibility is increased appreciably. The free energy cost of accessing some of these states from the intact nucleosome can be far less than the prohibitively high cost of unwrapping the entire DNA off the histone core. The availability of quantitative estimates of these costs is key to understanding of the nucleosome function. Below are several relevant examples.

Partial unwrapping of the DNA. The cost of unwrapping a ~ 10 bp long DNA fragment at each end is merely ~ 1 kcal/mol [38, 39], which means that the DNA in these regions becomes accessible with relatively high probability. Short fragments spontaneously unwrap and rewrap with high frequency [12, 34, 39], the corresponding life-times of the partially unwrapped states may be long enough to grant functional access to regulatory DNA target sites located there [12]. The free energy cost of unwrapping a single DNA fragment off the histone octamer increases roughly linearly with the fragment length, and thus the corresponding probability decreases exponentially [40, 41]; for DNA fragments deep inside the nucleosome the cost becomes substantial [2], *e.g.* $6-7$ kcal/mol for a fragment 70 bp away from the ends. The resulting partially unwrapped states are relatively long-lived [12], ~ 1 s. Finally, the free energy penalty for unwrapping long DNA fragments from both ends simultaneously might be higher than the sum for each fragment, especially once there is only a single turn left, as this turn does no longer feel an electrostatic repulsion from the other turn [42].

Partially Assembled Nucleosome Structures. Another mechanism that can facilitate access to the nucleosomal DNA is progressive disassembly of the histone octamer itself [15, 32, 35, 43, 44], which leads to the formation of partially assembled nucleosome structures (PANS), each lacking several histones. Importantly, thermodynamic parameters, such as apparent equilibrium constants, have been measured for several transitions between these states [45], which enables quantitative reasoning and modeling. A combination of Atomic Force Microscopy and Molecular Dynamics simulations reveals [11] atomistic details and dynamic aspects of some of the PANS, Fig. 2 (C), likely to occur on pathways of nucleosome assembly and disassembly. Despite the strong electrostatic attraction between the remaining histones and the DNA, a significant amount of the DNA remains free in each of the PANS [11]; for example in the tetrasome, $(\mathrm{H3{\cdot}H4})_2 \cdot \mathrm{DNA}$, about 78 bp of the DNA is accessible, by the above mentioned definition. The cost of removing H2A and H2B histones from the nucleosome to form the tetrasome is about 10 kcal/mol [45], on par with the cost of freeing up similar amounts of the DNA via partial unwrapping discussed above.

Post-translational modifications in the histone core. Yet another mechanism utilized by the cell to modulate the state of its chromatin, and cause a wide range of structural and biological responses, is reversible structural modifications to the histone proteins [46] such as acetylation, methylation, ubiquitination, crotonylation or phosphorylation, specific to certain amino-acids within the histone protein, Fig. 1 (B). The role of these post-translational modifications (PTMs) is extremely diverse. For example, some PTMs can act as markers for the binding of transcriptional factors [47]. Others, mainly located in the histone tails, and extensively studied, are implicated in affecting inter-nucleosomal interactions [48–52]

most relevant to the formation of nucleosome arrays, Fig. 1 (C). There also exists a class of PTMs that directly modulate the strength of association between the histone octamer and nucleosomal DNA [45, 53–56]; in this respect, PTMs that alter the charge of the nuclesome (acetylation, phosphorylation, crotonylation, propionylation, butyrylation, formylation, citrullination) are of particular interest, since electrostatics is the dominant interaction that governs the formation and stability of the nucleosome [16, 18, 27, 57]. For example, acetylation of H3K56 (Fig. 1 (B)), shown to increase transcription rates [46], results in a significant destabilization of the nucleosome, $\Delta\Delta G = 2.0$ kcal/mol [45]. A highly simplified physics-based model [16] pointed to a strong sensitivity of the nucleosome stability to the charge of the globular histone core, Fig. 2 (B), implying that charge-altering PTMs, such as lysine acetylation, in the globular core might be utilized by the cell as a mechanism of direct control of the DNA accessibility. Even though only a handful, out of hundreds possible, PTMs in the globular histone core of the nucleosome has been explored in functional essays, experimental evidence suggests that charge-altering PTMs can have significant biological consequences [46]. Taking into account atomistic details of the nucleosome and its partially assembled states enables to predict the effect of almost all unexplored (the vast majority) charge-altering PTMs in the globular core on the DNA affinity and its accessibility [31]. The general conclusion is consistent with the previous finding [16] based on a highly simplified geometry of the nucleosome – decreasing the charge of the globular histone core increases DNA accessibility. However, the additional realism of the new model leads to a more nuanced picture: the predicted effect of charge-altering PTMs varies dramatically, from virtually none to a strong, region-dependent increase in accessibility of the nucleosomal DNA upon PTM, Fig. 2 (D), hinting at the possibility of fine-tuning and selective control of DNA accessibility. Counter-intuitively, a few predicted acetylations, such as that of of H4K77, decrease the DNA accessibility [31], indicative of the repressed chromatin phenotype. Proximity to the DNA is suggestive of the strength of the PTM effect, but there are many exceptions [31]. Experimentally, PTMs in different regions of the histone core were shown to affect the nucleosome differently [58], *e.g.* acetylation of several lysines in the DNA entry-exit region, but not in the dyad region, promoted partial unwrapping of the DNA ends.

2.4. Nucleosome positioning

As mentioned above, DNA that is wrapped into a nucleosome is sterically occluded and typically not available to other DNA binding proteins such as transcription factors. Therefore, the positions of nucleosomes along DNA molecules can be of crucial importance. Most interestingly, the positions of many nucleosomes are not random. This can be seen by producing nucleosome maps using genome wide assays that extract DNA stretches which were stably wrapped in nucleosomes (see *e.g.* [59, 60]). For instance, nucleosomes are found to have a lower occupancy at functional binding sites of transcription factors than at non-functional sites [59].

What causes the non-random positions of nucleosomes? This is not straightforward to answer as there are many competing mechanisms at work. The nucleus contains not just DNA and histones, but also many other proteins that compete for binding to the DNA. In addition, chromatin remodellers hydrolyze ATP to actively push and pull nucleosomes along

DNA molecules. We focus here on yet another mechanism that is intrinsic to the interaction of DNA with histones and is mainly caused by the physical properties of the DNA molecule itself.

The sequence preferences of nucleosomes. The sequence preference can be demonstrated by reconstituting chromatin from its pure components, DNA and histone proteins [61]. Through salt dialysis the interaction strength between histones and DNA is gradually increased and eventually nucleosomes form. There are positions on the DNA where they form more likely than on average, so-called nucleosome positioning sequences. The preference of one sequence over another can be quantified by the difference in the affinities of the DNA stretches in question to the histone octamer, allowing to determine the *relative free energies* [14]. The sequence preference can be substantial, and comparable to the effect of some charge-altering PTMs: *e.g.* the artificial "high affinity" sequence 601 (discussed in more detail further below) has been reported to have a 2.89 kcal/mol lower free energy than the strong natural positioning sequence 5S of the sea urchin [14]. It is, however, worthwhile to mention that such affinity values have to be obtained under identical experimental conditions. A more recent study [45] using a different approach reported a much lower value of 0.7 kcal/mol.

When sequencing the stably wrapped DNA portions (after digesting the rest with micrococcal nuclease) one learns what types of base pair sequences cause higher-than-average affinities to nucleosomes, namely sequences where a larger than average number of particular base-pair steps are at certain positions on the nucleosome, see Fig. 3 [59, 62]. But what is precisely the mechanism that causes these sequence preferences? Is it mainly related to DNA mechanics and geometry or instead to some specific interactions between nucleobases and histones? A simple computational nucleosome model that mainly accounts for the sequence dependent elasticity and geometry of the DNA double helix does indeed predict the sequence preferences of real nucleosomes *in-vitro* [63], suggesting that the sequence dependent nucleosome affinity mainly reflects the ease with which DNA can be wrapped inside a nucleosome. We note, however, that the first-order elasticity approach used in this and many other studies to describe the strongly distorted DNA states inside nucleosomes is under debate as e.g. discussed in Ref. [64].

The *in-vitro* preferences carry over to some extent to nucleosome positioning *in vivo*. For instance, the characteristic dinucleotide preferences shown in Fig. 3 were already known to characterize stable nucleosomes extracted from chicken [62]. Such observations led the late Jonathan Widom and coworkers in 2006 [59] to propose a genomic code for nucleosome positioning, suggesting therefore that genomes have evolved to position nucleosomes. Building a probabilistic model trained on experimental nucleosome maps (of yeast or chicken) they noticed that they could predict the positions of a substantial (about 50%) fraction of nucleosomes in yeast. However, these claims have led to a major debate that has not subsided yet [65].

Yeast vs. humans. It becomes increasingly clear that the extent to which and the mechanisms by which sequence-dependent DNA elasticity determines nucleosome positions in living organisms vary vastly between species. We illustrate this by contrasting yeast [59, 61, 66] and

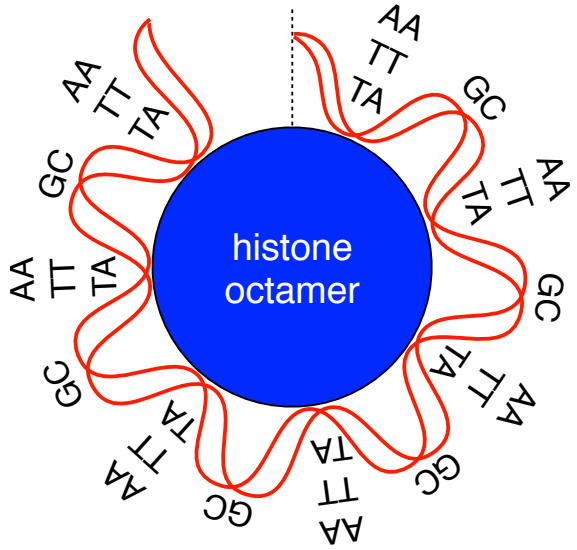

Figure 3: The nucleosome *in vitro* sequence preferences. High affinity sequences show more than on average GC steps (nucleotide G followed by nucleotide C) at positions where the major groove faces the histone octamer (every 10th bp) and TT, AA and TA step at positions where the minor groove faces the octamer [59, 62].

recent results from humans [67] and other higher vertebrates [68]. The nucleosome patterns around transcription start sites in yeast suggest a non-random ordering of nucleosomes, especially when looking at the genome-wide average. One can even count the nucleosomes that are positioned as one moves into the gene as +1 nucleosome, +2 nucleosome and so on [66]. But are these nucleosomes really positioned by dedicated mechanical signals on the DNA molecule?

As it turns out, yeast (and many other single-celled organisms [69]) feature, just in front of transcription start sites, regions characterized by a low content of Gs and Cs and the presence of A-tracts. Such sequences have a low affinity to nucleosomes and as a result act effectively as barriers to nucleosomes. Nucleosomes nearby (*e.g.* downstream of a transcription start site) are quite densely crowded and form, on average, a statistical pattern as they exclude each other. Such a statistical pattern close to a boundary constraint (in the current context provided by a stretch of stiff DNA repelling nucleosomes) has been already suggested by Kornberg and Stryer [70] and this mechanism might in fact be also largely responsible for the nucleosome positioning in yeast, at least close to transcription start sites. The claim in Ref. [59] that many nucleosomes in yeast are positioned mainly by the DNA sequence has therefore to be taken with a grain of salt, as there is not much indication of dedicated mechanical signals to position individual nucleosomes.

In contrast, in humans and other higher vertebrates the situation is rather different and much more in favor of the idea of dedicated mechanical cues. Audit, Arneodo and coworkers [67, 68] found well-positioned nucleosomes located around so-called nucleosome inhibiting barriers spread all over the genome of those organisms. The nucleosomes around those barriers are not just statistically ordered as in yeast, but instead they are positioned by

characteristic patterns of GC- and TA- rich regions. These nucleosomes alone contain about 30% of the nucleosomes mapped *in vivo* on the human genome. Even though the function of these nucleosomes is still unknown, these findings demonstrate that Widom's original claim might indeed be correct if applied to the right organisms.

2.5. Asymmetric nucleosomes

An interesting extension of the theme of the previous subsection is as follows: if some nucleosomes are positioned by mechanical cues at certain positions on a genome, then the DNA mechanics can also be used to equip these positioned nucleosomes with additional physical features. For instance, *Caenorhabditis elegans* shows typically (*i.e.* on a genome wide average) a positioned nucleosome directly downstream of the transcription start site [60, 69]. This nucleosome shows (on a genome wide average) a highly asymmetric sequence such that one half is much tighter wrapped than the other. The biological function of this built-in asymmetry is not clear yet, but it is worthwhile to mention that such asymmetric nucleosomes can act as polar barriers for elongating RNA polymerases [71].

New exciting experimental approaches allow to demonstrate directly the highly asymmetric nature of some nucleosomes as it results from an asymmetry of the underlying sequence. As it happens, the most popular DNA sequence for reconstituting nucleosomes, the Widom 601 sequence, is an example of a strongly asymmetric nucleosome. This sequence has been pulled out of a very large pool of random sequences for its strong affinity to histone proteins [72]. The Pollack group has recently demonstrated the highly asymmetric nature of that nucleosome, consisting just of the 601 sequence wrapped around the histone octamer (without linker DNA connecting to other nucleosomes). By performing small angle x-ray scattering on a solution of such particles with contrast variation (to render the protein cores invisible) they can observe a large ensemble of 601 nucleosomes that occur in various states of unwrapping [73, 74]. As mentioned above, thermally induced partial unwrapping of nucleosomal DNA is a mechanism through which DNA binding proteins can gain access to nucleosomal DNA, albeit with a much smaller equilibrium constant than for free DNA [20]. So far, one could only measure accessibility to a given DNA position inside the nucleosome, but the new method allows one to observe the whole breathing nucleosome. Importantly, it allows to distinguish the two ends of the nucleosomal DNA, since their mechanical properties differ and thus lead to different thermal fluctuations of the unwrapped portion. This feature enables the demonstration that the 601 nucleosome unwraps highly asymmetrically.

Another approach to study the asymmetric nature of the 601 nucleosome is micromanipulation together with FRET [75]. A single nucleosome was reconstituted on a longer DNA molecule containing one 601 positioning sequence, and then put under tension in a micromanipulation setup. At the same time the opening of one particular location was detected via FRET. This experiment demonstrated in great detail how an asymmetric nucleosome responds to external forces. It also showed how important sequence can be in determining the response. Whereas the original 601 nucleosome unwraps always from one end, the introduction of just three TA step on one half of the nucleosomal DNA (to make it more symmetric) leads to a nucleosome that unwraps with equal probability from either end.

2.6. Multiplexing genetic and mechanical information

Finally, we stress that mechanical cues that position nucleosomes and equip them with special physical properties are not restricted to be written on non-coding DNA stretches. As it turns out, coding DNA has enough wiggle room to contain a layer of mechanical information. This is a consequence of the degeneracy of the genetic code (64 codons encode for only 20 amino acids). Using a computational nucleosome model with sequence dependent DNA elasticity it was demonstrated that a positioning signal for a nucleosome can be placed anywhere on a gene with single base-pair resolution – by using only synonymous mutations[2] [63]. Likewise, nucleosomes with a wide range of stabilities against external forces could be engineered *in silico* on a piece of coding DNA, again by only making use of synonymous mutations [76].

3. Nucleosome arrays

Nucleosomes, which are more-or-less regularly spaced along the DNA molecule, can interact with each other to form the secondary level of the chromatin architecture, *i.e.* nucleosome arrays, Fig. 1 (C). Here we refer to structures made of a few to a few tens of individual nucleosomes (the physics of even larger chromatin structures is discussed in a recent review [77]).

3.1. Role of the tails

The positively charged terminal histone tails, Fig. 2 (a), play a critical role in the formation of nucleosome array structures [48, 50, 78]: the tails interact with the negatively charged DNA, the neighboring nucleosomes, and linker DNA. A long-standing unresolved question in the field is whether a “histone code" exists – that is whether each specific combination of PTMs conveys a distinct functional meaning, akin to the triplet genetic code of the DNA. A recent computational work [79] suggests that, in this respect, the effect of combined acetylations of H4 tail may be more analogous to a rheostat rather than to a "binary code": how many of the sites are acetylated maybe more important than which specific ones. On the other hand, certain acetylation sites, such as H4K16 discussed below, are known to “code for" strong and specific effect. Thus, the true picture is likely more nuanced, possibly including both cumulative non-specific and specific features.

3.2. The over-all structure

In contrast to the nucleosome, even the overall architecture of the nucleosome arrays is debated [80–82], let alone a fully atomistic description. For a while it was thought that a very regular type structure, the so-called 30 nm fiber [83, 84], was highly prevalent, but multiple recent lines of evidence call this view into question. For example, a study utilizing a novel electron microscopy-based methodology [85] concluded that chromatin is a flexible and disordered chain, ranging from 5 to 24 nm in diameter, with highly variable packing density

[2] A synonymous mutation in a DNA sequence is a mutation that does not change the encoded amino acid sequence. This can be achieved by swapping synonymous codons.

in the interphase nucleus. Despite this advance, the debate over exactly what the structure of chromatin is at truly *in-vivo* conditions will likely continue. What is certain is that nucleosome arrays take on many different, inter-converting structural forms [86, 87], which could be dependent on cell type and cell-cycle stage [88]. However, even in the absence of well-defined chromatin structures, basic physical principles, physics-based simulations and experiments contribute to the understanding of which structures are likely to occur under certain conditions, and how various biologically relevant modulating factors [87] affect transitions between different states of chromatin compaction, Fig. 1. Several approaches exist for making the structure-to-function connection at this level [89], including a version [85] of the DNA accessibility argument.

3.3. DNA condensation by oppositely charged particles

One fruitful physics-based approach to understanding chromatin structure at this nucleosome array level is based on the idea that the basic physics [90] that governs condensation of the self-repelling DNA by oppositely charged particles is universal, and therefore applies to nucleosome arrays as well [19, 91]. The physics of nucleic acid condensation by polyions is indeed relatively well understood by now [19, 90, 92–94]. In particular, the majority of the DNA charge must be neutralized for the remaining charge-charge repulsion to be weak enough for the condensation to occur [90]. Since, in the case of the nucleosome, the histones (including the tails) neutralize only about 50% of the nucleosomal DNA, a significant portion of the negative DNA charge must be neutralized by other readily available positively charged entities [19], including Mg^{++}, linker histones, protamines, basic domains of the nuclear proteins, polyamines, etc. The state of chromatin at physiological conditions appears to be "nearly condensed", close to the phase boundary separating it from states of much looser compaction [19]. This "nearly condensed" state of chromatin is maintained by a tightly controlled balance between some of the modulating factors: the amount of the core histones, linker histones, and nucleosome repeat length [95, 96]. Even minor alterations of the delicate charge balance, such as acetylation of a single lysine (K16) on the H4 histone tail, may lead to chromatin de-compaction [97], which, in turn, leads to transcription activation [98]. The de-compacting effect on chromatin structure of reducing the positive charge of the histone tails is consistent with the general picture of DNA condensation governed by a subtle interplay between charge-charge repulsion, ion-ion correlations, and, in the case of the nucleosome arrays, histone-tail bridging that facilitate formation of the folded/aggregated structures [52].

A nuanced picture. While the most general physical principles behind chromatin condensation at the nucleosome array level may be well understood, the detailed picture of nucleosome array condensation/de-condensation is highly nuanced. For example, the effect of charge-altering post-translational modifications on the array compaction varies widely, even within the same histone tail: the effect of H4K16 acetylation on the array unfolding is much stronger than that of H4K12, H4K8 or H4K5 [52]. The specific strong effect of H4K16 acetylation may be due to its role of promoting tail-mediated nucleosome-nucleosome stacking [52]. Simulation reveals [48] that H4K16 is the only acetylation site interacting with the acidic patch

on the neighboring nucleosome; its acetylation disrupts the electrostatic interactions of K16 that favor array compaction. And even that detailed picture may be more nuanced still [99].

From the point of view of its function – providing on-demand access to the genomic information – it makes sense that condensed chromatin at physiological conditions should be near the phase boundary separating the condensed from the looser, less condensed states where the DNA is easily accessible. Similar to the case of the nucleosome reviewed above, Fig. 2 (B), the state of chromatin condensation is then easy to control by small, physiologically meaningful adjustments to relevant modulating factors.

4. Conclusions

In this brief review we have offered an opinion that physics-based methods, approaches and reasoning are very useful tools in understanding the complexity of chromatin structures, making structure-function connections, and generating experimentally verifiable predictions. Especially approaches based on thermodynamics, classical electrostatics, and physics-based simulations – well-established in the field of traditional structural biology of proteins and DNA – can also be quite useful in the emerging field of structure-based epigenetics. For reasons of space, the examples we chose to support our opinion are limited to the primary (the nucleosome) and the secondary (nucleosome arrays) level of the chromatin structural hierarchy.

A general picture that emerges is that the state of chromatin at physiologically relevant conditions is close to a "phase boundary" separating compact, dense structures where accessibility to genomic DNA is significantly restricted, from looser structures with increased DNA accessibility. Higher accessibility generally means enhancement of processes that depend on it, such as transcription. The closeness of chromatin to the "compact-loose" phase boundary facilitates on-demand fine-tuning of the DNA accessibility by the cell. Bringing in more details, including atomistic ones, allows for more detailed predictions, such as the role of specific post-translation modifications of the histone proteins or sequence effects of the wrapped DNA on the stability of nucleosomes.

While evidence of success of the physics-based approaches in the field is growing, one also becomes aware of their inherent limitations. Predictions of good models can be expected to provide correct trends and guidance for future experiments usefully above the Null model levels, but one can not expect in this field the spectacular level of accuracy and reliability that physics delivers for the hydrogen atom or planetary motion. Evolution, the Blind Watchmaker, does not necessarily choose the most mathematically elegant or simple solutions so appealing to a physicist – these can sometimes fail spectacularly when checked against biological reality [100].

Declarations of interest: none.

Acknowledgements

A.V.O. thanks Nikolay Korolev for many helpful suggestions, discussions, and a stimulating argument; and Andrew Fenley for commenting on the manuscript draft. Partial

support from the NSF (MCB-1715207) to A.V.O. is acknowledged.

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