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The impact of the sequence-dependent physical properties of DNA on chromatin dynamics

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Abstract

The local mechanical properties of DNA depend on local sequence. Here we review recent genomic, structural, and computational efforts at deciphering the "mechanical code", i.e., the mapping between sequence and mechanics. We then discuss works that suggest how evolution has exploited the mechanical code to control the energetics of DNA-deforming biological processes such as nucleosome organization, transcription factor binding, DNA supercoiling, gene regulation, and 3D chromatin organization. As a whole, these recent works suggest that DNA sequence in diverse organisms can encode regulatory information governing diverse processes via the mechanical code.

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Introduction

Big strides in molecular biology have been marked by advances in our understanding of how DNA sequence encodes information. That DNA sequence encodes protein-coding information was fueled by early seminal works such as the solving of the DNA structure, the decipherment of the genetic code, and the establishment of the central dogma of molecular biology. Simultaneously, the idea that stretches of special recognition sequence motifs along DNA can encode regulatory information by recruiting trans-acting regulatory factors gained traction: the early discovery of the TATA box as a core promoter element that binds the TATA binding protein (TBP) [1], the discovery of the mechanism of regulation of the lac operon [2], and subsequent discoveries of myriad promoters, enhancers, or transcription factor binding sites have all contributed to this understanding. Later still, from around the 1980s onwards, it was discovered that the recruitment of trans-acting factors to DNA is further modulated by the state of DNA methylation, with significant consequences for gene regulation and cell differentiation [3]. Thus, epigenetic modifications of DNA bases were revealed to be yet another means by which DNA encodes information. More recently, the idea that sequence can encode regulatory information by controlling the shape and mechanical properties of chromatin at various scales has gained traction. Three general observations suggest this view: (1) almost all known processes involving DNA such as DNA:protein interactions [4], DNA supercoiling, or DNA packaging, involve some mechanical distortions of DNA such as bending, twisting, stretching, or supercoiling [5], (2) DNA deformations cost energy because DNA has measurable mechanical properties such as persistence length or torsional rigidity, that allow it to resist deformations [6,7], and (3) the local mechanical properties of DNA are variable, depending on local sequence [6,8]. Thus, sequence, via its effect on the mechanical properties of DNA, can potentially have a regulatory effect on the myriad critical biological processes that require DNA deformations. This review will focus on recent developments that highlight how DNA can mechanically encode regulatory information in certain selected contexts.

Sequence dependence of the mechanical properties of DNA

Substantial evidence has been gathered to suggest: (i) the existence of a "mechanical code", i.e., a mapping between local DNA sequence and the local mechanical properties of DNA; and (ii) that evolution may have taken advantage of the mechanical code to select for local sequences with specific mechanical properties to regulate biological processes that require DNA deformations.

Various physical properties of DNA, such as mechanical flexibility, shape, melting temperature, or propensity to form plectonemes, are impacted by chemical interactions between individual bases and thus depend on local sequence. Interactions between bases include base pairing and hydrogen bonding between bases on

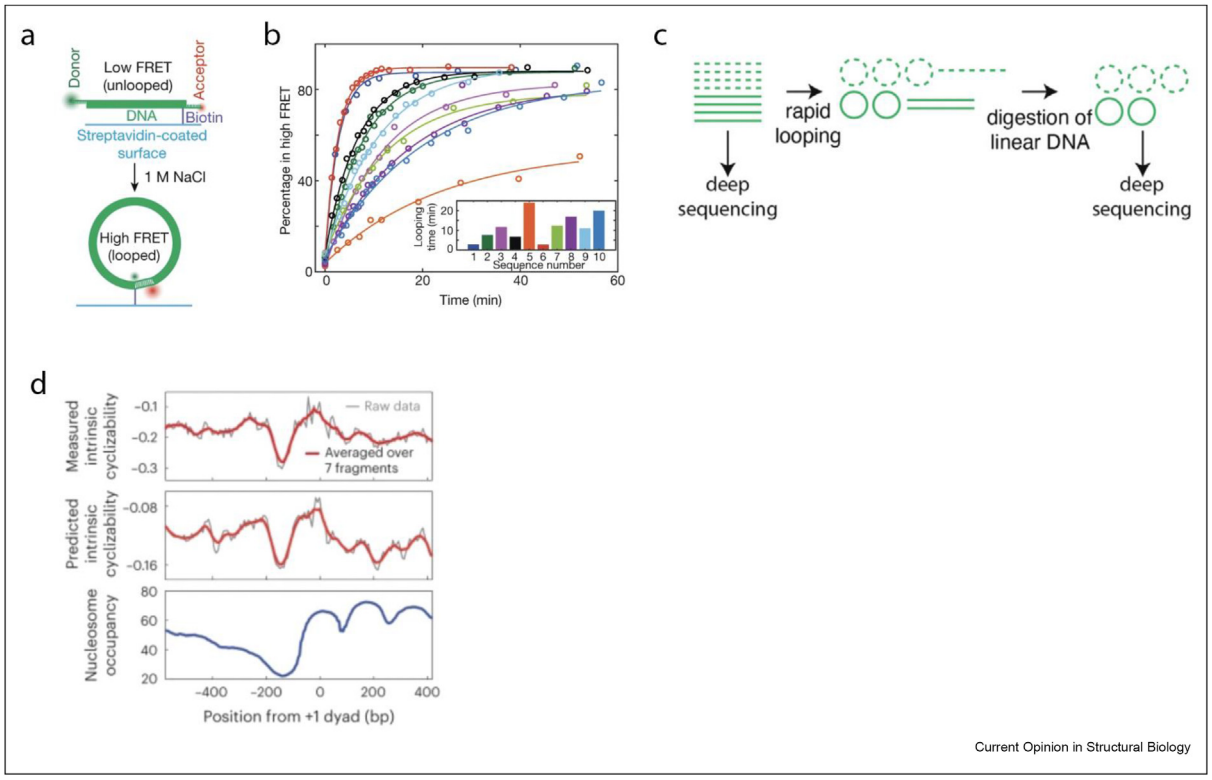
complementary strands and base stacking interactions involving van der Waals forces between the aromatic rings of adjacent bases on a single strand [9]. Differences in base pairing interactions between AT and GC base pairs are directly reflected in the dependence of DNA melting temperature on GC content [10]. Differences in local DNA shape have also been linked to structural differences in the interactions between bases. Olson and coworkers compiled the structures of various DNA sequences in available DNA:protein crystal structures and quantified how local DNA sequence impacts local DNA shape parameters (like twist, roll, and tilt) and the energy function for fluctuations about the mean shape [11]. Pyrimidine-purine dimers, and particularly the TpA dimer, were identified as acting like flexible hinges. Such sequence-dependent variations in DNA shape parameters are linked to overall mechanical flexibility and curvature. An early example was the poly-A tract, which was shown in crystal structures to be straight and rigid [12]. A high degree of propeller twist (i.e., high deviation from coplanarity of bases within a base pair) within the dA-dT tract was seen to be present, which enhances stability by increasing purine-purine base-stacking interactions [12] and allowing for an additional system of bifurcated hydrogen bonding. Structural analysis also revealed a high degree of roll (angular deviation of DNA about its long axis), which accumulates in phase if the tract is repeated at the helical pitch, leading to overall curved DNA [12]. Subsequently, sequence-dependent, intrinsically curved DNA has been observed in many instances to serve biological functions, an early compilation of which can be found in the introduction section of this reference [13].

In addition to observing static DNA structures, dynamic experiments have played a big role in deciphering the sequence dependence of DNA flexibility. Early experiments involved performing DNase I digestion of DNA minicircles, which were used to quantify how each dinucleotide or trinucleotide step contributes to cutting efficiency and, by proxy, minor groove width and bending stiffness [14,15]. The bending propensity data from trinucleotide contributions were shown to mimic the observed local roll angles in various protein:DNA crystals. DNA cyclization experiments that measure the propensity of a short DNA duplex flanked by complementary single-stranded overhangs to undergo intramolecular cyclization have long been used to measure the mechanical flexibility or bendability of the fragment in question. Such measurements have been performed on a limited set of short, 200 bp DNA sequences, to determine how dinucleotide steps contribute to DNA persistence length [16]. The data are consistent with TA dinucleotides being very flexible and CG dinucleotides being very rigid. More recently, single-molecule fluorescence resonance energy transfer (smFRET)-based DNA looping assays were used to measure the kinetics of DNA cyclization on the mesoscale of about 100 bp [17] (Figure 1a). The authors observed that such fragments can readily loop despite being shorter than the persistence length of DNA (~150 bp [18]). Looping can, however, be attributed to nonsmooth bending modes such as kinking, base flipping, and melting [19] or to the mechanical properties of the 10 nucleotide single-stranded overhangs on either end of the duplex [20], all of which would be wholly consistent with the known persistence length of DNA. Thus, the term looping encompasses any mode of DNA distortion that brings distal points along DNA into proximity, as is often required for the formation of various DNA:protein complexes. Single-molecule looping [17,19] showed that looping times of different sequences can vary by more than an order of magnitude (Figure 1b), once again demonstrating the strong sequence dependence of the dynamic flexibility of duplex DNA.

A recent approach to improving the decipherment of the "mechanical code" involved carrying out looping measurements on a large number of DNA sequences to establish general rules that map sequence to DNA cyclizability. A technique called loop-seq was developed to accomplish this (Figure 1a), which has been described and reviewed in detail earlier [6,19,21]. Briefly, DNA molecules in a large library containing multiple copies of as many as ~100,000 different ~100 bp DNA sequences flanked by complementary single-stranded overhangs is briefly allowed to undergo intramolecular cyclization. Unlooped molecules are enzymatically digested, while looped molecules are preserved, thus enriching the library for the more flexible sequences. The original library and the selected library are subject to deep sequencing. The ratio of the relative population of each sequence in the selected library to that in the original library is calculated and used as a measure of cyclizability or bendability.

Recent works have used cyclizability measurements obtained via loop-seq to attempt to decipher the mechanical code [22,23]. It was found that the overall GC content of a DNA fragment does not contribute to its cyclizability. However, the number of times individual dinucleotides and tetranucleotides occur in the fragment was shown to be correlated with cyclizability [22]. In particular, TpA dinucleotides were shown to be associated with flexible DNA, consistent with several other reports that TpA might serve as a flexible hinge [11,16,24]. CpG dinucleotides were associated with rigid DNA, consistent with earlier measurements based on systematic evolution of ligands by exponential enrichment (SELEX) [25]. In addition, the manner in which dinucleotides are distributed along a sequence was also found to impact cyclizability in a quantifiable and predictive manner. Essentially, short A/T or G/C rich stretches were suggested to curve DNA when present at the helical repeat and straighten it when present at half

Figure 1



(**a**) Schematic of the single-molecule DNA cyclization assay. Panel reproduced with permission from this reference [19]. (**b**) Looping kinetic curves showing the percentage of molecules in the looped state as a function of time since the addition of 1M NaCl (which starts the process of looping). All molecules are initially prepared in the unlooped state. Different colors reflect kinetic curves for different sequences. Inset: Looping times (obtained by fitting the kinetic curves to single exponentials and extracting the time constant) of the 10 sequences. Panel reproduced with permission from this reference [19]. (**c**) Schematic of the loop-seq assay. For demonstration, the initial library contains just two different DNA sequences (dashed and continuous) and only four copies of each sequence. The results of deep sequencing will indicate that the dashed sequence is relatively more enriched in the selected library as compared to the original library and is thus more cyclizable. (**d**) Measured (via loop-seq [6]) and predicted (via the physical model developed on the basis of loop-seq data [22]) intrinsic cyclizability of DNA along all annotated genes in chromosome V of yeast. The bottom panel also shows the independently measured nucleosome occupancy [64].

the helical repeat. This is consistent with earlier structural studies that identified such short sequences as capable of bending DNA towards the minor or major grooves respectively [16,26,27]. The bends thus add in phase or cancel out when repeated at the helical or half-helical periods respectively. These observations were used to develop both machine learning and correlative models for the sequence dependence of DNA cyclizability [22].

Molecular dynamics simulations have revealed more subtle aspects of the sequence dependence of DNA bendability — tightly bent DNA configurations, such as in minicircles, undergo "inside-out" conformational transitions, with the more likely configurations being determined by sequence and methylation state. The work found that minicircles comprise straight segments interspersed by bends that compress the inward-facing major groove and thereby favor configurations where stiffer base pair sequences avoid such a compressed major groove [28].

Impact of sequence-dependent DNA mechanics on chromatin dynamics

Recent developments in characterizing the sequence-dependence of DNA mechanics have made it possible to understand the impact of sequence-encoded variation in DNA mechanics on diverse chromatin transactions. Here we discuss a few select examples that have been recently investigated.

Nucleosomes form ubiquitously along the entire length of eukaryotic genomes. Each nucleosome involves the tight wrapping of 145–147 bp DNA around an octamer of histone proteins [29]. Nucleosomes compact the genome and prevent aberrant transcription [30]. This makes it imperative for cells to keep the region of DNA immediately upstream of transcription

start sites (TSSs) nucleosome-free, to allow proper assembly of the transcription machinery. Additionally, promoter proximal nucleosomes just downstream of the TSS enable proper transcription (rather than repress it), as these nucleosomes can bear important post-translational modifications and otherwise contribute to stages in transcription initiation and elongation. Thus proper positioning of nucleosomes, especially around TSSs, is critically important for cell function. It therefore raises the question of whether the sequence-dependent mechanical properties of DNA play a role in properly positioning nucleosomes around TSSs.

Consistent with nucleosomes involving extensive DNA bending, various experiments involving forming nucleosomes on special DNA sequences have highlighted that nucleosomes form better on flexible DNA substrates, and vice versa: (1) sequences known to prefer a specific curvature direction maintain that direction when incorporated into nucleosomes [31,32], (2) specially-designed bendable sequences form nucleosomes more efficiently [32], and (3) sequences selected for nucleosome formation efficiency show evidence of greater bendability [33]. Sequence-dependent energy functions for DNA bending, obtained from compiled crystal structure data, have been used to predict the propensity of various sequences to form nucleosomes [34], suggesting that sequence-dependent DNA flexibility plays a role in regulating the formation of highly bent DNA-protein complexes.

Beyond studying nucleosome formation on isolated short DNA sequences, several studies have investigated how sequence, through its impact on DNA flexibility and shape, can determine nucleosome positioning genome-wide. A physical model in which both DNA elastic energy and histone-DNA interaction terms were used to calculate the penalty of deviation of nucleosomal DNA from an ideal superhelix was successfully used to predict *in vitro* nucleosome positioning [35]. Another physical model that takes into account both bending and shearing deformations of DNA predicted nucleosome occupancy *in vitro* and *in vivo* and suggested the dominance of shearing deformation energy in nucleosome positioning [36]. By isolating yeast nucleosomal DNA and analyzing the sequences, Segal et al. constructed a nucleosome-DNA interaction model and used it successfully to predict 50% of *in vivo* nucleosome positions [37]. More recently, loop-seq was used to map DNA cyclizability along an entire chromosome in yeast [19]. When compared to known nucleosome positioning data [38], it confirms that nucleosomes, chromosome-wide, tend to form in regions of flexible DNA and avoid rigid DNA regions (Figure 1d). Nucleosome-depleted promoter regions were found to be unusually rigid as compared to neighboring regions, while regular arrays for gene-body nucleosomes were found to be centered on corresponding regions of flexible DNA. Moreover, the choice of codons along gene body nucleosomes was shown to have been optimized by evolution to establish the pattern of DNA flexibility variations conducive to nucleosome organization. Similar patterns of sequence-encoded DNA cyclizability as measured by loop-seq, correlating with nucleosome occupancy, have been reported in other species as well, like Drosophila and mouse [22,23].

Although accumulated evidence suggests a role for DNA bendability in nucleosome positioning, it is worth noting that the 601 DNA sequence, which very strongly positions nucleosomes *in vitro* [25], does not show strong nucleosome positioning *in vivo* when inserted in the yeast genome. Future analysis that compares loop-seq data on DNA bendability along yeast genes with *in vitro* nucleosome positioning data on yeast genomic DNA [39] (as has been obtained via salt-gradient dialysis in the absence of any other DNA-binding factor) might serve to better determine the extent of the causal role of DNA bendability in positioning nucleosomes.

The discussion on nucleosome positioning thus far has focused mainly on translational positioning — the location of nucleosome dyads along the genome. The exact position of a nucleosome within the helical repeat of DNA is referred to as its rotational positioning, and earlier evidence suggests a role for DNA sequence, particularly the positions of specific dinucleotides, in nucleosome rotational positioning [40]. It is possible that sequence-dependent DNA curvature (rather than dynamic flexibility) could favor a specific rotational positioning that aligns the curvature direction with the curvature of the dyad axis of DNA along the nucleosome. Indeed, examples of how A/T or G/C-rich short nucleotide stretches bend DNA towards the minor or major grooves and lead to overall curved molecules when repeated at the helical pitch have been wellstudied in previous works [16,26,27]. In loop-seq-based measurements of DNA cyclizability, a similar "rotational" effect impacting cyclizability has been observed — the location of the biotin tether that attaches the looped molecule to the bead surface imparts a phase term to cyclizability that oscillates at the helical repeat. This is likely because cyclizability has a contribution from the intrinsic curvature of DNA, and tether orientations that allow the looped molecule to curve away from the surface (as opposed to curve towards it) would favor looping. This has been explained in detail in supplementary note 7 of this [19] reference. Current loop-seq analysis averages out this phased contribution by taking measurements at various biotin tether locations. Though speculative, it may be possible in future analyses or experiments to explicitly use this effect to report on the sequence-dependent contribution to the rotational positioning of nucleosomes genome-wide.

ATP-dependent chromatin remodelers have long been known to play a major role in positioning and spacing nucleosomes, especially around critical loci such as TSSs. As DNA mechanics has also been suggested to influence nucleosome positioning, it raises the question of whether nucleosome remodelers use or override the information in sequence-dependent DNA mechanics to properly position nucleosomes [41]. In 2007, Rippe et al. observed the nucleosome sliding activities of seven different nucleosome remodelers and showed that DNA sequence plays a role in determining the remodeled state of nucleosomes [42]. In particular, for the remodeler ACF (ATP-utilizing chromatin assembly and remodeling factor), a DNA sequence element that positions nucleosomes was identified, and it was shown that nucleosomes, once formed on this sequence, show reduced affinity for subsequent translocation. A similar mechanism was suggested for the remodeler Chd1.

More recently, *in vitro* reconstitution of nucleosomes on genomic DNA in the presence of various purified chromatin remodelers and other factors was used to show that the chromatin remodeler INO80, even in the total absence of any other factor, can correctly position the +1 nucleosome (the first nucleosome downstream of the TSS) and deplete nucleosomes upstream of the TSS [39]. The implication, therefore, was that INO80 must detect some feature of DNA sequence around TSS, and this was suggested to be the local sequence-dependent helical twist. Via loop-seq, a sharply defined region of rigid DNA found ubiquitously at yeast promoters. It was speculated to possibly provide a barrier to the nucleosome translocation activity of INO80 [19], thereby allowing downstream nucleosomes to stack against the barrier while depleting nucleosomes upstream. It was later confirmed from structural studies that INO80 requires bending of extranucleosomal DNA, consistent with the idea that regions of very stiff DNA will pose a barrier to INO80 translocation [43,44]. Direct experimental confirmation of whether the rigid DNA region at promoters can impede DNA bending by INO80 and whether this subsequently prevents nucleosome translocation will require future experiments. The idea that DNA mechanics might impact other remodelers in identifying and positioning promoter proximal nucleosomes has also been suggested in the context of the remodeled SWR1 [45].

Although many studies have focused on nucleosome organization around transcription start sites, loop-seq has recently been used to probe the role of nucleosome organization around a different sort of locus. The binding site for the transcription factor CTCF [46] has been shown to facilitate the formation of well-ordered nucleosomal arrays on either side, while the site itself may be occupied by a fragile nucleosome [47,48]. Both predictive models [23] and direct loop-seq measurements [22] have confirmed the presence of sequence-encoded local peaks in DNA cyclizability at and around CTCF binding sites in mouse embryonic stem cells, co-centric with known nucleosome positions, suggesting that sequence-encoded DNA mechanics might have evolved to facilitate nucleosome organization around CTCF binding sites.

Predictive models for DNA cyclizability have recently been used to suggest a wider role of DNA mechanics in diverse biological processes that involve DNA bending, extending beyond nucleosome dynamics. For example, sequence-encoded DNA cyclizability might impact the DNA supercoiling activity of the topoisomerase DNA gyrase [22]. In addition, the location of DNA plectonemes that are generated as a result of supercoiling has been shown to be pinned by the sequence-dependent local geometric properties of DNA [49], and in turn likely regulates transcription. In fact, the expression level of a large fraction of the genome is regulated by the overall genomic superhelical density in complex ways [50,51], though no mechanism for how overall supercoiling up-regulates some promoters and down-regulates others has been found. Certain sequence features have been identified in these categories of promoters, suggesting a possible role for the sequence-dependent physical properties of DNA [50] in creating a dependence of promoter expression level on superhelical density.

The role of DNA mechanics in impacting transcription factor (TF) binding efficiency was recently probed by a high-throughput method called saturation mismatch-binding assay (SaMBA) [52]. TF binding ubiquitously involves extensive DNA deformations [5]. For each transcription factor studied, a library of all possible single mismatches in a 60 bp DNA fragment surrounding its known binding sites was generated. Fluorescently labeled transcription factors binding to members of this library were quantified to measure equilibrium dissociation constants. The authors showed that mismatches, which can significantly alter local DNA mechanics and structure, can provide part of the energetic penalty for the transcription factor to properly distort DNA. These observations raise the possibility that sequence-encoded variations in DNA mechanics may also have been exploited by evolution to regulate TF binding dynamics, though verification must await future experiments.

The mechanical properties of DNA on the mesoscale might impact the local 3D architecture of chromatin. The recent development of techniques such as Hi-CO [53] and RICC-seq [54] have provided unprecedented 3D maps of nucleosome positioning and orientation on the scale of a few nucleosomes. Special chromatin folds on the tetranucleosome scale have been identified as being associated with, or depleted at, transcription start and end sites, suggesting a functional relevance associated with transcription [55]. Future works that integrate

Hi-CO or RICC-seq data with the sequence dependence of DNA bendability and torsional rigidity can likely reveal how sequence-encoded mechanical properties of DNA can accommodate the required bends and twists of linker DNA in order to attain specific functional 3D arrangements of nucleosomes [55].

It is possible that local DNA mechanics and nucleosome organization might impact higher-order chromatin structure as well. Structural maintenance of chromosomes (SMC) proteins play a fundamental role in organizing higher-order chromatin structure. Well-known examples of SMCs, such as cohesins, compact DNA via loop-extrusion [56,57]. It is possible that loop-extrusion initiation, which requires significant local DNA bending, may be regulated by the local physical properties of DNA as determined by sequence, epigenetic modifications, or even DNA damage. This is, however, purely a conjecture and requires experimental testing. Likewise, the formation of plectonemes as a result of negative supercoiling of the bacterial genome has long been suggested to both globally compact chromatin and regulate gene expression [50,51]. Where and to what extent supercoils partition into plectonemes depends on the local relative energetic contribution of DNA bendability and torsional rigidity. This in turn may both be encoded in sequence via a mechanical code, as has recently been demonstrated [49].

Very recently, the mechanical code was shown to be modulated by the state of DNA methylation [22,58]. Cytosine methylation in the CpG context is a major means of gene regulation in multicellular organisms [59]. Developmental programs and diseases like cancers are known to alter gene expression by altering CpG methylation patterns [59]. A major way in which CpG methylation impacts downstream processes is undoubtedly via the recruitment of special transcription factors that recognize it [59]. However, it has long been suggested that CpG methylation might also impact gene expression by altering the physical properties of chromatin [58,60–62]. Recently, the introduction of CpG methylation in yeast, which natively lacks it and thus also lacks transcription factors that recognize it, was shown to still lead to several of the phenotypes associated with CpG methylation in mammals [63] such as low levels of CpG methylation at start sites of highly transcribed genes. Loop-seq measurements on DNA libraries with methylated CpGs suggested that CpG methylation decreases the dynamic flexibility of DNA and buffers against the intrinsic curvature induced by CpG dinucleotides by preventing DNA from bending towards the major groove [22]. Direct measurements suggested that CpG methylation around TSSs in mouse would alter the pattern of DNA bendability. This may alter either downstream nucleosome positioning or the action of chromatin remodelers, although confirmation must await future experiments. Nevertheless, it raises the possibility that part of the downstream biological effects of developmental programs or diseases that alter the epigenetic landscape of DNA may be achieved via the impact such alterations have on the physical properties of chromatin. The ongoing understanding of how the sequence-dependent physical properties of DNA may have been exploited by evolution to encode regulatory information will likely impact both our understanding of and ability to control diverse DNA transactions.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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