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A molecular view of DNA flexibility

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Review

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Abstract

DNA dynamics can only be understood by taking into account its complex mechanical behavior at different length scales. At the micrometer level, the mechanical properties of single DNA molecules have been well-characterized by polymer models and are commonly quantified by a persistence length of 50 nm (~150 bp). However, at the base pair level (~3.4 Å), the dynamics of DNA involves complex molecular mechanisms that are still being deciphered. Here, we review recent single-molecule experiments and molecular dynamics simulations that are providing novel insights into DNA mechanics from such a molecular perspective. We first discuss recent findings on sequence-dependent DNA mechanical properties, including sequences that resist mechanical stress and sequences that can accommodate strong deformations. We then comment on the intricate effects of cytosine methylation and DNA mismatches on DNA mechanics. Finally, we review recently reported differences in the mechanical properties of DNA and double-stranded RNA, the other double-helical carrier of genetic information. A thorough examination of the recent single-molecule literature permits establishing a set of general 'rules' that reasonably explain the mechanics of nucleic acids at the base pair level. These simple rules offer an improved description of certain biological systems and might serve as valuable guidelines for future design of DNA and RNA nanostructures.

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Introduction

The physical properties of DNA are interrogated in virtually every process that involves storage or manipulation of the genetic information encoded in the double-helix. DNA packaging

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inside eukaryotic nuclei requires bending of the DNA around the histone octamer; and gene regulation in bacteria often involves DNA looping in regulatory regions. Transcription and DNA replication require local melting of the double-helix and produce a torsional stress in the DNA that is absorbed via the formation of plectonemes. In the pursue of a quantitative characterization of these and other biological processes, intensive research has been devoted to study the mechanical properties of DNA (Bloomfield *et al.*, 2000). In particular, in the last few decades, our understanding of DNA mechanics has been propelled by the advent of single-molecule techniques, which enable manipulating and/or measuring individual DNA molecules. Initial single-molecule experiments on DNA stretching and supercoiling (Smith *et al.,* 1992; Strick *et al.*, 1996) showed that the mechanical properties of long (several kilobase pairs) DNA molecules are well described by polymer models that neglect the microscopic details of the duplex (Marko and Siggia, 1994, 1995*a*, 1995*b*). Since then, these polymer models have resulted extremely useful to quantify, for instance, the action of proteins on the DNA, and are currently employed to calibrate single-molecule biophysical instruments on a daily basis (Bouchiat *et al.*, 1999; Bustamante *et al.*, 2003; Madariaga-Marcos *et al.*, 2018).

Despite the unquestionable success of polymer models, recent single-molecule assays are encouraging an alternative, i.e. microscopic description of DNA flexibility (Wiggins *et al.*, 2006; Lipfert *et al.*, 2010; Vafabakhsh and Ha, 2012; Lebel *et al.*, 2014; Shon *et al.,* 2019; Pyne *et al.*, 2020). Ultimately, DNA is a highly sophisticated molecule with fine molecular features such as major and minor grooves, stacking interactions, or hydrogen bond donor and acceptors, that are inextricably linked to the flexibility of the double-helix. For example, dinucleotides that present weak stacking interactions are prone to induce a sharp bend in the DNA (Olson *et al.*, 1998); sequences with a narrow minor groove resist mechanical deformations (Nelson *et al.*, (1987); and exotic hydrogen bonds found in certain sequences can assist complex deformations of the double-helix (Dans *et al.*, 2014; Pasi *et al.*, 2014). Consequently, despite behaving as a homogeneous polymer at long scales, DNA presents an intricate flexibility at short distances that is strongly dependent on the nucleotide sequence (see Fig. 1). In addition, non-canonical DNA base pairs including e.g. methylated cytosines or mismatched base pairs, can substantially alter the mechanics of DNA at such short scales (see Fig. 1). Thus, given that DNA:protein interactions usually occur at the nanometer scale, it is paramount to deepen the molecular description of DNA flexibility, which will possibly provide additional insights into biological phenomena beyond the reach of classical long-range polymer approaches. Moreover, a molecular characterization of DNA mechanics may pave the way for expanding the nanotechnological applications of DNA as building material.

Here, we review recent studies that are advancing our understanding of single-molecule DNA mechanics from such a molecular perspective. We focus on two approaches that are driving major progress in this field. First, the development of new single-molecule assays that permit assessing novel mechanical properties of DNA, especially at short-length scales. Second, the synergistic combination of single-molecule experiments and computer simulations, most notably molecular dynamics (MD), that model the mechanics of DNA with atomic details. This review aims to discuss recent studies that illustrate the capability of these two approaches at providing novel relevant insights into double-stranded DNA mechanics. Thus, it is not our intention to deepen in any particular aspect of DNA mechanics or to include non-helical DNA structures, such as G-quadruplexes or i-motifs (see Abou Assi *et al.*, 2018; Mandal *et al.*, 2019 for reviews on these topics).

This review has been divided into three sections. In Section 'Sequence-dependent DNA mechanics', we comment on how the nucleotide sequence affects the physical properties of DNA. We review sequences that facilitate or preclude DNA bending; sequences prone to adopt a double-helical structure that differs from the canonical B-DNA; and sequences where DNA melting is more favorable. In Section 'Chemical modifications and DNA mechanics', we discuss the effect of modifications of the canonical duplex, in particular, methylated cytosines and DNA mismatches. In Section 'Mechanical properties of dsRNA: unexpected differences with dsDNA', we cover recent studies that are revealing interesting differences between the mechanics of DNA and double-stranded RNA (dsRNA), the other double-helical carrier of genetic information that is found in nature. Finally, we present the conclusions of the review and suggest future lines of research.

The findings on DNA mechanics hereby reviewed have often provided a new perspective on diverse biological systems, as discussed throughout the text. An exciting challenge for the near future will be to exploit our ever-growing knowledge on nucleic acids mechanics for controlled design of functional DNA and RNA nanostructures.

Sequence-dependent DNA mechanics

In single-molecule experiments, the mechanical properties of DNA are commonly quantified by means of its persistence length, P_{DNA}. If one thinks of a long shoelace or a (cooked) spaghetti immersed in a swimming pool, one does not picture a perfectly straight line, but rather, a somewhat coiled object. The same occurs for DNA. The size of the DNA polymer in solution, as given by the distance between its two ends, is quantified by P_{DNA}. An alternative – perhaps more intuitive – way of looking at P_{DNA} is the threshold DNA length above which the molecule will start to bend. In other words, DNA molecules shorter than P_{DNA} are expected to be essentially straight. Several single-molecule experimental setups, including magnetic tweezers (MT), optical tweezers (OT) (Smith *et al.*, 1992, 1996; Bustamante *et al.*, 1994; Wang *et al.*, 1997), and atomic force microscopy (AFM) imaging (Rivetti *et al.*, 1996; Heenan and Perkins, 2019), allow extracting the value of P_{DNA} with high accuracy. These and many other single-molecule techniques yield, under standard buffer conditions, a consensual value of P_{DNA}~50 nm, which corresponds to ~150 bp (e.g. the length of DNA in the nucleosome core).

Note, however, that the accepted value of P_{DNA}~150 bp contrasts with several biological evidences where the DNA is severely bent at distances comparable to or shorter than 150 bp. Examples of such bends include DNA loops at regulatory regions or the wrapping of nucleosomal DNA around the histone core (reviewed in Garcia *et al.*, 2007). According to the commonly accepted worm-like chain (WLC) model, such short-scale deformations would be prohibitive for a DNA molecule with P ~150 bp.

This conundrum motivated studies on the local mechanics of single DNA molecules (Wiggins *et al.*, 2006; Vafabakhsh and Ha, 2012). Such studies revealed a striking ability of the duplex to adopt strongly bent conformations, an aspect that was not contemplated by the standard WLC model (Wiggins *et al.*, 2006; Vafabakhsh and Ha, 2012). Although the quantitative aspects of short-scale DNA bending remain a subject of debate, it is clear

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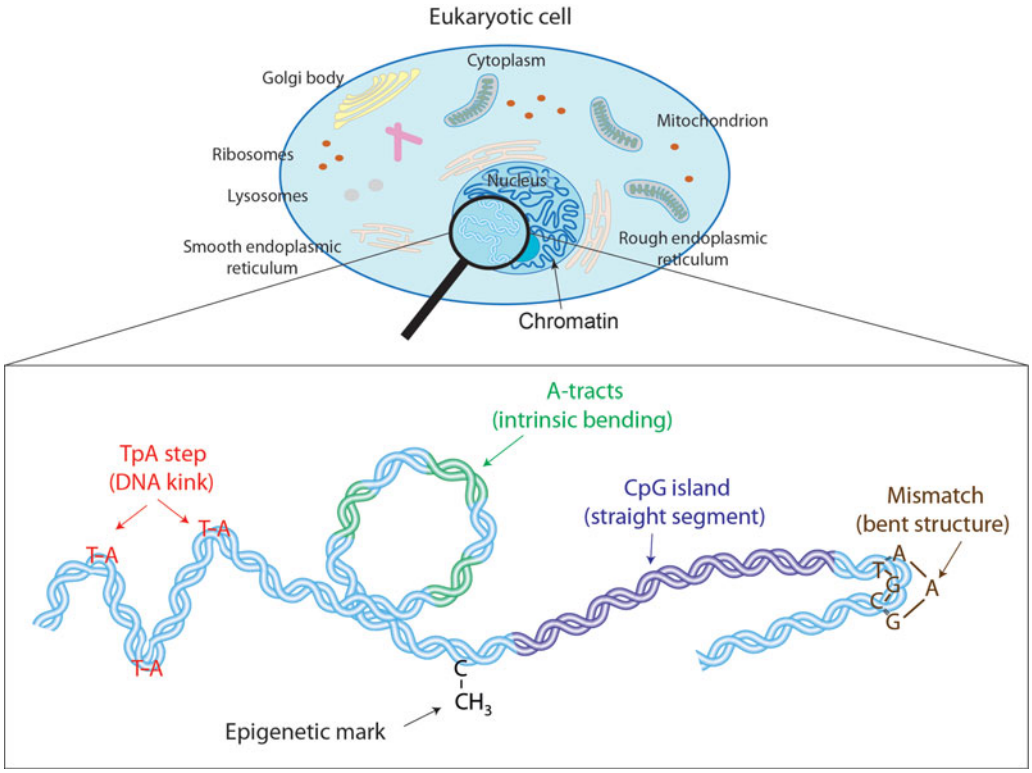


Fig. 1. Effectors of DNA mechanical properties at short-length scales. DNA is highly compacted in the chromatin inside the nucleus of a cell. We will show along the text that multiple effectors have been described to modulate the DNA mechanical properties at short-length scales. These examples comprise kinkable TpA steps, intrinsic bending by A-tracts, rigid CGIs, mismatches that produce strong bending, and cytosine methylation that exert a versatile role in DNA physical properties.

that sharp DNA bending can proceed via concerted localized distortions of the duplex, such as DNA kinks (Vologodskii and Frank-Kamenetskii, 2013). More interestingly, the nucleotide sequence strongly affects these local deformations (Vafabakhsh and Ha, 2012), in contrast to the long-range persistence length, which shows little sequence-dependent variation (Geggier and Vologodskii, 2010). Namely, sequence effects on DNA mechanics appear amplified at short-length scales.

TpA dinucleotides are highly flexible

DNA kinks were predicted long ago (Crick and Klug, 1975) and have been observed in crystal structures of a number of DNA:protein complexes (Berman *et al.*, 1992; Olson *et al.*, 1998), including nucleosomal DNA (Olson and Zhurkin, 2011); and also in several MD simulations (Lankaš *et al.*, 2006; Curuksu *et al.*, 2009; Irobalieva *et al.*, 2015; Harrison *et al.*, 2019). The biological consequences of DNA kinks are diverse, and have been reviewed in the context of DNA wrapping in the nucleosome core (Richmond and Davey, 2003; Olson and Zhurkin, 2011) and of specific DNA:protein interactions (Rohs *et al.*, 2010). Crystal structures of DNA:protein complexes reveal that kinks are more favorable in pyrimidine-purine steps, CpG and CpA, and particularly in the TpA step, which has shown larger flexibility (Olson *et al.*, 1998) (see Table 1 for nomenclature). However, structural studies provide a static description of the DNA and thus, are of limited use in order to address the dynamics of kink formation.

The development of novel single-molecule assays to interrogate the flexibility of short DNA molecules is a promising tool to probe the impact of kinkable pyrimidine-purine steps on the physical properties of DNA. Of particular relevance is the creative single-molecule assay developed by Vafabakhsh and Ha (2012). By attaching a pair of fluorescence resonance energy transfer (FRET) dyes to DNA molecules and measuring the FRET signal, the authors were able to observe single-molecule DNA cyclization in real time (see Fig. 2*a*). In that study, the authors showed that the cyclization dynamics, as quantified by the *J*-factor, was much faster than the WLC prediction, supporting the existence of strongly bent DNA structures, as proposed in a previous AFM study (Wiggins *et al.*, 2006).

More importantly, this single-molecule cyclization assay was employed to explore the effect of the nucleotide sequence on DNA flexibility. In a later study, the same group studied the cyclization dynamics of the two halves of the 601 Widom sequence (a strong nucleosome positioning sequence) (Ngo *et al.*, 2015). They found that the left half, which contains four kinkable TpA steps, was highly flexible, whereas the right half, which only contains one of such steps, was relatively rigid (see Fig. 2*a*). Notably, when this rigid half was modified to include three additional TpA steps, it became much more flexible, albeit it was still more rigid than the left, flexible half. This experiment shows that even few TpA steps can substantially impact the flexibility of DNA molecules comprising tens of base pairs. Further single-molecule studies should aim to address whether this effect is also present in CpG and CpA steps, which have also been traditionally identified as highly flexible (Olson *et al.*, 1998).

A deeper understanding of the physical properties of DNA sometimes offers a fresh perspective on certain biological processes. This is well illustrated in the study by Ngo *et al.* (2015). Besides the aforementioned cyclization experiments, the authors

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Table 1. Nomenclature used for DNA and dsRNA sequences

Term	Definition
NpM step	A dinucleotide of sequence 5′-NM-3′, e.g. TpA denotes 5′-TA-3′
Poly(dN:dM)	DNA sequences consisting of several consecutive nucleotides, e.g. poly(dA:dT) denotes sequences such as 5′-AAAAAAA-3′
Poly(dN–dM)	DNA sequences consisting of alternating N and M nucleotides, e.g. poly(dA–dT) denotes sequences of the form 5′-ATATATATA-3′ or 5′TATATAT-3′
A-tract	DNA sequences with three or more consecutive adenines and/or thymines, without the flexible TpA step. Examples are 5′-AATT-3′, 5′-TTTTTT-3′, but NOT 5′-TTAA-3′ (which contains a TpA step)
CpG island	DNA sequences with high proportion of CpG steps, e.g. 5′-ACGAGCGGCGTCG-3′ or 5′-CGTCGAGCGTCGGGCG-3′
AU-tract	dsRNA sequences consisting of several (three or more) alternating adenines and uracils, e.g. 5′-rArUrArUrArU-3′ or 5′rUrArUrA-3′

Note: The sequences shown here refer to a canonical Watson–Crick double-helix where the reverse strand is omitted for clarity, e.g. the sequence 5′-AGTACCC-3′ refers to a DNA double-helix with forward strand 5′-AGTACCC-3′ and reverse strand 5′-GGGTACT-3′. Ribonucleotides are referred to as rA, rC, rU, and rG.

used a combined setup of OT and single-molecule FRET (smFRET) to observe single unwrapping events in nucleosomes. This novel assay permits visualizing which side of the nucleosomal DNA is detached from the histone core when tension is applied. They observed that reconstituted nucleosomes with the aforementioned 601 sequence showed highly asymmetric unwrapping. In particular, upon the action of an external force, the right rigid half of the nucleosomal DNA was significantly more prone to unwrap than the left, flexible half. On the contrary, nucleosomes reconstituted with the 601 sequence containing additional TpA steps in the right half showed an approximate symmetric unwrapping. Namely, when the two halves of nucleosomal DNA had a similar flexibility, nucleosome unwrapping occurred stochastically from either side. Since the publication of the study by Ngo *et al.* (2015), a number of experimental and computational studies have addressed the link between DNA flexibility and transient nucleosome unwrapping, or nucleosome breathing (Lequieu *et al.*, 2016; Zhang *et al.*, 2016; Chen *et al.*, 2017; Culkin *et al.*, 2017; Mauney *et al.*, 2018; Winogradoff and Aksimentiev, 2019). These studies have revealed that, besides its well-known role in positioning nucleosomes (Widom, 2001; Segal *et al.*, 2006; Kaplan *et al.*, 2009), the DNA sequence can largely affect the accessibility of certain regions within nucleosomal DNA via changes in the flexibility of the duplex.

A dual role of A-tracts in DNA flexibility

In contrast to the flexibility of TpA steps, other DNA sequence motifs are thought to be extremely rigid. The most well-known examples are A-tracts, a kind of DNA sequence that consists of three or more adenines and thymines without the flexible TpA step. Poly(dA:dT)s, a particular case of A-tracts, possess a strong nucleosome depleting character that has been associated to a presumably high rigidity of this motif, as predicted from structural studies and atomistic simulations (Nelson *et al.*, 1987; Haran and Mohanty, 2009; Segal and Widom, 2009; Geggier *et al.*, 2014).

The flexibility of A-tracts in general, and poly(dA:dT)s in particular, are controversial, because they are inextricably intertwined with the long-known intrinsic bending induced by these sequences (see, e.g. Zhang and Crothers, 2003; Thompson and Travers, 2004; Haran and Mohanty, 2009; Peters and Maher, 2010; Ortiz and de Pablo, 2011). For example, previous AFM and tether particle motion experiments indicated that A-tracts have no enhanced bending rigidity, showing a standard persistence length of ∼50 nm (Rivetti *et al.*, 1998; Brunet *et al.*, 2015*a*). However, these results contrast with coarse-grain simulations and single-molecule cyclization experiments, which suggest that A-tracts are rigid to bending (Vafabakhsh and Ha, 2012; Mitchell *et al.*, 2017).

In a recent study, these paradoxical mechanical properties of A-tracts were comprehensively studied at the single-molecule level using a wealth of techniques: MT, OT, and AFM imaging in air and in liquid (Marin-Gonzalez *et al.*, 2020*a*). The authors considered a sequence from the *Caenorhabditis elegans* genome with several repetitions of phased A-tracts, expected to display a strongly bent character (Fire *et al.*, 2006). Indeed, AFM measurements demonstrated that phased A-tracts induce an intrinsic bend in the DNA that could be well-described with a variant of the WLC model that includes intrinsic bending (see Fig. 2*b*) (Rezaei *et al.*, 2018; Marin-Gonzalez *et al.*, 2020*a*). Moreover, both AFM and OT measurements independently showed that, at long-length scales, the A-tract molecule was not particularly rigid (nor flexible) to bending, showing a persistence length of 54 nm. However, as the A-tracts were subjected to high forces ($F > 10$ pN), an extraordinary rigidity started to emerge, that was quantified by a large stretch modulus (see Fig. 2*b*). Such high forces are expected to align the intrinsic A-tracts bends, thus enabling to probe the local rigidity of the A-tract structure. It was thus proposed that, although A-tract might not seem rigid at long distances, the stiff local structure of the A-tract would resist short-scale mechanical deformations, which are likely more determinant for nucleosome formation.

We can thus conclude that A-tracts play a dual role in DNA flexibility. On the one hand, they induce a static bending in the double-helix, but on the other hand the structure of the A-tract itself appears rigid. It is tempting to state that the balance of these two effects can be regulated by the distribution of A-tracts along a given DNA region. That is, several short A-tracts in phase with the helical pitch would amplify the bending, whereas a long (i.e. >20 bp) individual A-tract would stiffen the DNA. This intriguing dual mechanism appears to be exploited *in vivo* in the context of nucleosome positioning: short, phased A-tracts are enriched in nucleosome positioning sequences Rohs *et al.* (2009), whereas long poly(dA:dT)s are characteristic of nucleosome depleting regions (Segal and Widom, 2009).

CpG islands are rigid DNA regions depleted of nucleosomes

Another example of DNA motif whose biological functions might depend on peculiar mechanical properties are CpG islands (CGI),

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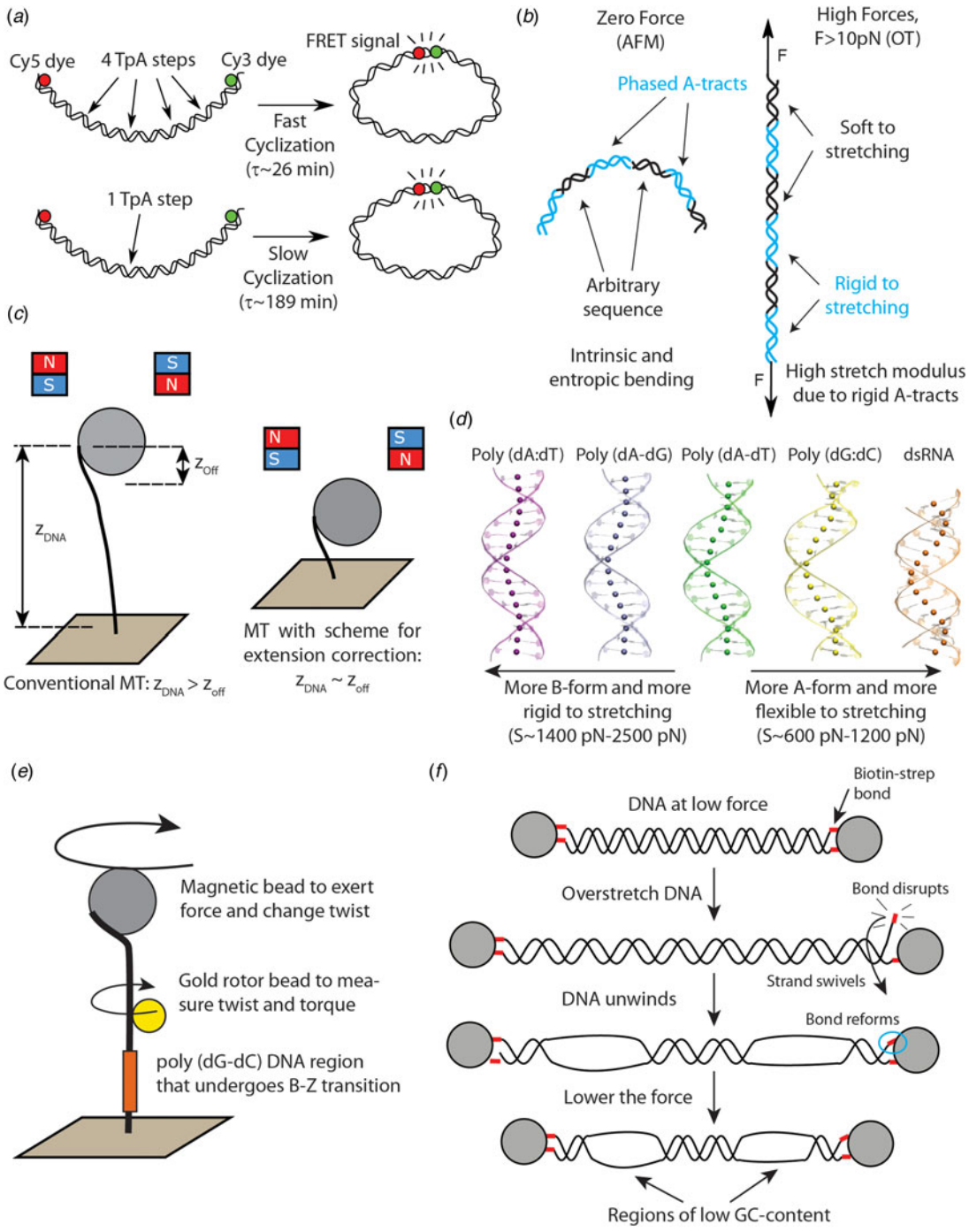


Fig. 2. Recent single-molecule and MD studies on sequence-dependent DNA mechanics. (*a*) The single-molecule assay developed by Vafabakhsh and Ha (2012) consists of a pair or FRET dyes attached to the extremes of a DNA molecule with cohesive ends. Cyclization results in an increase in the FRET signal, thus enabling measuring single-molecule cyclization in real time. TpA steps were found to increase DNA flexibility, resulting in faster cyclization kinetics Ngo *et al*. (2015). (*b*) Cartoon illustrating the complex mechanical properties of A-tract sequences. A-tracts located in phase with the DNA helical pitch induce a global macroscopic curvature in the molecule. When the molecule is subjected to high forces, the bends are straightened and the A-tracts are found to present a large stretching rigidity. Thus, A-tracts induce DNA bending, but the tracts themselves are rigid at a local level. Adapted from Marin-Gonzalez *et al*. (2020*a*). (*c*) MT are usually limited to measurements of DNA molecules with contour length longer than the bead radius. This limitation is overcome in the MT scheme for extension correction developed in Shon *et al.* (2019), which enables estimating the anchor point of the DNA on the bead. Using this correction scheme, accurate MT force–extension curves can be obtained for DNA molecules as short as ~200 bp. (*d*) The crookedness curvature reported in Marin-Gonzalez *et al*. (2019*a*) is responsible for sequence-dependent variations of the DNA extension and distinguishes between A- and B-DNA conformations. Curved sequences, such as poly(dG:dC) are A-like and flexible to stretching; straight sequences, such as poly(dA:dT) are highly B-like and rigid to stretching. Adapted from Marin-Gonzalez *et al*. (2019*a*). (*e*) The gold rotor bead assay from Lebel *et al*. (2014) combines high-resolution torque spectroscopy and controlled stretching and supercoiling of DNA molecules. A magnetic bead permits exerting force and supercoiling DNA molecules; whereas a gold bead attached to the side of the molecule reports on its twist and torque. This system has been used to study the B–Z transition of a poly(dG–dC) sequence with high temporal resolution. (*f*) Assay for DNA supercoiling using OT, as reported in King *et al*. (2019). Upon overstretching, one of the biotin–streptavidin interactions that attach the DNA to the optical beads is disrupted. The DNA unwinds and then the bond reforms, trapping the DNA in a negatively supercoiled state. When the force is lowered, melting bubbles are observed in regions of low GC-content.

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GC-rich DNA regulatory regions with a high proportion of CpG steps. In vertebrates, CGI are ubiquitous sites of transcription initiation, a feature that has been linked with a deficient assembly of CGI into nucleosomes (both *in vivo* and *in vitro*) (Ramirez-Carrozzi *et al.*, 2009; Deaton and Bird, 2011). As for poly(dA:dT), it is likely that the depletion of nucleosomes at CGI is related to a peculiar rigidity of these sequences (Marin-Gonzalez *et al.*, 2019*a*; Shon *et al.*, 2019; Pongor *et al.*, 2017).

Recent developments in single-molecule techniques have enhanced our understanding of the mechanical properties of CGI. Shon *et al.* (2019) developed a novel scheme for *in-situ* correction of the DNA extension in MT, which enables obtaining accurate force–extension curves of DNA molecules as short as 198 bp. This development extends the capability of conventional MT, which are usually limited to DNA molecules longer than ~1 kbp; and thus optimizes this technique for better exploration of sequence effects on DNA flexibility (see Fig. 2*c*). Using their novel correction scheme, Shon *et al.* found that a CGI DNA molecule possessed a large persistence length when compared with a control DNA of arbitrary sequence, indicative of a high bending rigidity of CGI. Interestingly, this and other studies have proposed that the flexibility of CGI is highly sensitive to the methylation state of these sequences (Marin-Gonzalez, 2019*a*; Shon *et al.*, 2019; Pongor *et al.*, 2017), suggesting that epigenetic marks might exert regulatory functions in CGI via changes in the mechanical properties of the DNA. This will be discussed in Section 'The impact of cytosine methylation on DNA physical properties'.

Despite these insightful studies, further inquiry is clearly needed to better understand the mechanical properties of CGI. First, there is some discrepancy among different single-molecule techniques regarding the large rigidity of CGI (Pongor *et al.*, 2017), which should be solved in future studies. Such studies should take into account that CGI not only have a high GC-content, but also a high density of CpG steps. In fact, it is expected that the frequency and distribution of CpG steps would play a more relevant role in the mechanics of DNA than the GC-content itself (Marin-Gonzalez *et al.*, 2019*a*). In parallel to single-molecule studies, computer simulations should aim at explaining the molecular mechanisms behind the proposed rigidity of CGI. This is a challenging task, given the complex deformability exhibited by the CpG step, which is highly anharmonic and dependent of its sequence context (Dans *et al.*, 2014).

Poly(dA–dT) and poly(dG:dC) adopt A-like DNA conformations

In addition to DNA flexibility, the nucleotide sequence can strongly impact the conformation of the double-helix. In particular, some sequences, such as poly(dG:dC), have been identified to possess structural features close to the A-form (A-philic), whereas others such as poly(dA:dT) are often classified as highly B-like (B-philic) (Lu *et al.*, 2000). Yet, another class include poly(dA–dT) sequences, which adopt a so-called TA-DNA structure, or hybrid between B- and A-forms (Lu *et al.*, 2000; Kulkarni and Mukherjee, 2017). This rich conformational landscape of the double-helix is crucial for achieving DNA sequence recognition by several proteins that read specific features of the B- or A-forms (Lu *et al.*, 2000; Rohs *et al.*, 2010) (see Table 2).

Several structural parameters have been proposed as a measure of the A-philicity of a given DNA sequence, i.e. of its propensity to assume an A-like conformation (Lu *et al.*, 2000; Waters *et al.*, 2016; Kulkarni and Mukherjee, 2017). However, calculating these parameters requires knowledge of the atomic structure of the molecule, which is often inaccessible to single-molecule experiments. Is it possible to assess the A-philicity of a DNA sequence without knowing its atomic structure?

Recently, a promising approach has been proposed. Inspired by previous studies on dsRNA (Chou *et al.*, 2014; Lipfert *et al.*, 2014), Marin-Gonzalez *et al.* (2019*a*) suggested a connection between the A-philicity of a DNA sequence and its stretching flexibility. The authors performed MD simulations of several DNA sequences and they observed significant variations in the extension from one sequence to another. They noticed that these changes in the extension were a consequence of a sequence-dependent DNA curvature, denoted *crookedness*, that reasonably correlated with the A-philicity of the sequence (see Fig. 2*d*). For example, the poly(dG:dC), which is highly A-philic, showed a short extension and large *crookedness* curvature; whereas the highly B-philic poly(dA:dT) was essentially straight. Interestingly, those DNA molecules that were more crooked, and more A-like, were more flexible to a stretching force; and *vice versa*, highly B-like sequences were more rigid. Among the latter are poly(dA:dT) and poly(dC–dG), whose large stretching rigidity are supported by recent experiments on A-tracts and CGI (Pongor *et al.*, 2017; Marin-Gonzalez *et al.*, 2020*a*).

It is thus tempting to state that the stretching flexibility of a given DNA sequence is a good indicator of its A-philicity. This hypothesis is supported by naïve intuition. Note that a perfectly straight textbook B-DNA helix should be extremely hard to stretch. In such an ideal B-DNA, elongations beyond the contour length should result in unwinding and unstacking of the base pairs, which would easily disrupt the double-helix. This was already realized by Bustamante and coworkers in their seminal single-molecule MT experiment, where they proposed that the stretching elasticity of DNA should be a consequence of a 'local curvature of the DNA axis' (Smith *et al.*, 1992). It is plausible that such 'local curvature' consists of sequence-dependent deviations from the straight B-form into a more curved A-form (see Fig. 2*d*).

However, further research is needed to better characterize the DNA A-philicity, *crookedness* and stretching flexibility, in order to establish a more solid connection between these DNA features. Along this line, a recent study by Lankas and coworkers has expanded our understanding on DNA crookedness, by assessing how this parameter depends on the temperature of the system (Dohnalová *et al.*, 2020).

Poly(dG–dC) are hotspots of Z-DNA formation

Besides the right-handed helical structures discussed above, DNA is able to assume, under certain conditions, a left-handed conformation with a zig-zag backbone known as Z-DNA (Rich and Zhang, 2003). This DNA structure has been observed at sequences of alternating purines and pyrimidines, most notably poly(dG–dC), and is thought to require high salt concentrations or negative torsional stress in order to form (Rich and Zhang, 2003). The structural features of Z-DNA (and also Z-RNA) are recognized *in vitro* by certain proteins (those containing the so-called Zα domain, see Table 2). However, the *in vivo* relevance of Z-DNA is debated (Rich and Zhang, 2003; Herbert, 2019; Jiao *et al.*, 2020). In this regard, some studies point toward transient formation of Z-DNA as regulatory mechanism during

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Table 2. Sequence-dependent DNA physical properties

Sequence motif	DNA physical properties	Reference	Biological implications	Reference
Pyrimidine-purine steps (mainly TpA)	Highly flexible	Ngo *et al.* (2015)[a] Olson *et al.* (1998)	Stabilize nucleosomes, DNA:protein recognition via kinks	Ngo *et al.*(2015)[a] Lowary and Widom (1998); Widom (2001); Rohs *et al.* (2010)
Short (∼4–10 bp) phased A-tracts	Intrinsic bending B′-DNA with narrow minor groove	Rivetti *et al.* (1998; Moreno-Herrero *et al.* (2006)[a] Koo *et al.* (1986); Nelson *et al.* (1987); Haran and Mohanty (2009)	Stabilize nucleosomes, Promote supercoiling, DNA:protein recognition via minor groove electrostatics	Kim *et al.* (2018*b*)[a] Rohs *et al.* (2009); Rohs *et al.* (2010)
Long (>∼10 bp) poly(dA:dT)s	Very rigid	Vafabakhsh and Ha (2012); Marin-Gonzalez *et al.* (2020*a*)[a]	Destabilize nucleosomes	Field *et al.* (2008; Kaplan *et al.* (2009); Segal and Widom (2009)
CGI (rich in CpG steps)	Rigid	Shon *et al.* (2019);Pongor *et al.* (2017)[a] Marin-Gonzalez *et al.* (2019*a*)[b]	Destabilize nucleosomes	Ramirez-Carrozzi *et al.* (2009); Deaton and Bird (2011)
Poly(dG:dC)	A-like conformation	(Lu *et al.* (2000); Kulkarni and Mukherjee (2017) Marin-Gonzalez *et al.* (2019*a*)[b]	DNA:protein recognition, e.g. by zinc finger proteins	Lu *et al.* (2000); Rohs *et al.* (2010)
Poly(dA–dT)	TA-DNA conformation	Lu *et al.* (2000); Kulkarni and Mukherjee (2017) Marin-Gonzalez *et al.* (2019*a*)[b]	DNA:protein recognition, e.g. by TATA-binding protein	Lu *et al.* (2000); Rohs *et al.* (2010)
Poly(dG–dC)	B–Z transition under negative torsional stress	Lee *et al.* (2010*a*); Lebel *et al.* (2014); Bryant *et al.* (2012); Oberstrass *et al.* (2012, 2013)[a]	DNA:protein recognition via $Z\alpha$ domain, e.g. in ADAR	Rich and Zhang (2003)
GC-content	Explains melting in long (kbp) DNA molecules, but not in short ones	Huguet *et al.* (2010); Gross *et al.* (2011); King *et al.* (2019)[a] Sutthibutpong *et al.* (2016)[b]	DNA unwinding at the onset of replication or transcription	Gai *et al.* (2010); Larson et al. (2011)

[a]Single-molecule experiments.
[b]Computer simulations.

transcription, where the negative supercoils generated by RNA polymerase would stabilize this left-handed structure (Rich and Zhang, 2003).

A number of single-molecule studies have provided a quantitative description of the thermodynamics of Z-DNA formation (Lee *et al.*, 2010*a*; Lebel *et al.*, 2014; Bryant *et al.*, 2012; Oberstrass *et al.*, 2012, 2013). In a pioneer study, Lee *et al.* (2010*a*) combined MT and smFRET to simultaneously induce and measure B–Z transitions in a DNA molecule containing a $(dG–dC)_{11}$ sequence, prone to assume a Z-form. These single-molecule experiments indicated that, in the presence of tension, Z-DNA formation requires only a small torsional strain, much lower than predictions based on bulk experiments. The study by Lee *et al.* thus suggested that Z-DNA might occur *in vivo* more often than expected, given that tension is likely to accumulate on DNA, e.g. during transcription.

The dynamics of the B- to Z-DNA transition have also been studied using the rotor bead tracking assay (Oberstrass *et al.*, 2012, 2013; Lebel *et al.*, 2014; Lipfert *et al.*, 2015). In this experimental setup an extreme of the DNA is bound to a magnetic bead that permits stretching and supercoiling the duplex; and a second bead is attached to the side of the DNA at an intermediate position. The position and fluctuations of the latter bead inform, respectively, on the twist and torque of the system (Bryant *et al.*, 2003; Gore *et al.*, 2006*a*, 2006*b*) (see Fig. 2*e*). Using different versions of this rotor bead assay, Oberstrass *et al.* obtained torque-twist measurements on DNA molecules with 22 and 50 bp-long GC repeats and observed transitions consistent with the formation of a Z-DNA structure at the poly(dG–dC) regions (Oberstrass *et al.*, 2012, 2013). These measurements were able to recapitulate relevant features of the B–Z transition, such as its high cooperativity. Recently, rotor bead assays using a gold probe have allowed a more detailed characterization of this phenomenon, unveiling the B- to Z-DNA transition with unprecedented temporal resolution (Lebel *et al.*, 2014).

Altogether, single-molecule assays have provided a rather comprehensive description of the B- to Z-DNA transition at GC repeats under torsional stress. Future single-molecule studies should aim to explore the dynamics of this transition at other repeating sequences, and the impact of Z-DNA binding proteins on this process. Recent experimental studies are starting to provide interesting insights into these questions (Kim *et al.*, 2018*a*).

In addition to single-molecule experiments, MD simulations have also explored the B- to Z-DNA transition, shedding light on the atomistic mechanisms behind it (Lee *et al.*, 2010*b*; Moradi *et al.*, 2013; Chakraborty and Wales, 2017). Of particular relevance is the study by Moradi *et al.* (2013), which suggested that the B- to Z-DNA transition can proceed by means of several different mechanisms, rather than via a single pathway. In that study, MD simulations revealed a rich diversity of non-canonical DNA structures – such as an overstretched-like S-DNA, extensive base flipping or unpeeling of the two DNA strands – that act as intermediate states in the B- to Z-DNA transition. Future computational efforts should further elucidate the molecular aspects of this enigmatic process. Recent refinements of DNA force-fields which focused on improving the description of the Z-DNA structure (Zgarbová *et al.*, 2015) will offer an invaluable tool toward achieving this goal.

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GC-content not always explains DNA melting

Negative torsional stress regulates access to the genetic information. DNA unwinding promotes disruption of the base pairing interactions, or DNA melting, resulting in larger exposure of the nucleobases. DNA is thus unwound by specialized enzymes that locally denature the double-helix in order to read its nucleotide sequence, e.g. in transcription (Larson *et al.*, 2011), replication (Gai *et al.*, 2010), or CRISPR-mediated bacterial immunity (Szczelkun *et al.*, 2014). In addition, DNA melting often occurs as a consequence of *in vivo* torsional strains, and can result in the formation of particular structures such as kinks or bubbles that are specifically recognized by proteins (Fogg *et al.*, 2012).

At a macroscopic level (several kbp), DNA melting can be well explained from the GC-content of a given sequence (Marmur and Doty, 1962; Vologodskii and Frank-Kamenetskii, 2018). Namely, higher GC-content sequences better resist melting due to the three hydrogen bonds of the G:C base pair compared to the two bonds of the A:T base pair. This idea is supported by a recent single-molecule study that employs a novel creative assay to generate negative supercoils on DNA using a conventional OT set-up (King *et al.*, 2019) (see Fig. 2*f*). In typical OT experiments, the DNA molecules are attached to the optically trapped beads by means of biotin–streptavidin bonds. King *et al.* found that, when torsionally constrained molecules are overstretched (at forces of ∼110 pN), one of the biotin–streptavidin interactions can temporarily disrupt. When this occurs, the torsional stress accumulated on the DNA during overstretching can be partially relieved, by swiveling one DNA strand around the other (see Fig. 2*f*). Eventually, the broken biotin–streptavidin bond forms again, locking the DNA molecule in a negatively supercoiled state. The authors exploited this discovery to controllably unwind the DNA and induce melting events that were then detected using fluorescently labeled RPA protein (which strongly binds single-stranded DNA) as a reporter. Their results indicate that the sites of DNA melting are reasonably correlated with DNA regions of low GC-content. This finding is in agreement with experiments on DNA overstretching, where the thermodynamics of sequence-dependent DNA unpeeling could be predicted on the basis of the GC-content (Gross *et al.*, 2011).

At the microscopic level (few base pairs), however, torsion-induced DNA melting remains poorly understood. For example, studies on single-molecule unzipping of DNA hairpins suggest that, in such short DNA molecules, GC-content might not be a determinant factor of DNA melting (Huguet *et al.*, 2010; Camunas-Soler *et al.*, 2016; Vologodskii and Frank-Kamenetskii, 2018). Instead, stacking interactions (which are not directly related to the GC-content; Kilchherr *et al.*, 2016) can play an even more important role in DNA melting than base pairing interactions (Huguet *et al.*, 2010; Camunas-Soler *et al.*, 2016; Vologodskii and Frank-Kamenetskii, 2018). However, DNA melting under torsion is likely to be even more complex than DNA unzipping, and might depend on additional sequence-dependent molecular features such as DNA flexibility (Vlijm *et al.*, 2015; Shepherd *et al.*, 2020).

DNA minicircles offer an attractive platform for studying supercoiling-induced DNA denaturation at the molecular level. DNA minicircles consist of small (few hundreds of base pairs) closed DNA molecules that are subjected to high bending and torsional stress. Computer simulations have revealed a rich structural diversity in DNA minicircles, including kinks, base flipping, and denaturation events (Lankaš *et al.*, 2006; Irobalieva *et al.*, 2015; Sutthibutpong *et al.*, 2016; Pyne *et al.*, 2020). In a recent study, Sutthibutpong *et al.* combined atomistic MD, coarse-grained simulations and statistical mechanics calculations to study sequence-dependent melting in DNA minicircles (Sutthibutpong *et al.*, 2016). Coarse-grained techniques identified AT-rich regions to be more prone to undergo melting. Nevertheless, detailed atomistic MD simulations indicated that both breathing and melting events were more frequent in flexible pyrimidine-purine dinucleotides such as TpA or CpA (see Section 'TpA dinucleotides are highly flexible').

The complex interplay between base pairing, base stacking interactions and DNA flexibility in DNA unwinding remains an open question. We believe that the combination of MD simulations and high-resolution AFM imaging on DNA minicircles can be an extremely useful approach toward gaining such molecular understanding of sequence-dependent DNA melting. Ongoing research in this direction is already yielding very promising results (Pyne *et al.*, 2020).

Chemical modifications and DNA mechanics

In the previous section, we have discussed how the nucleotide sequence impacts the flexibility of canonical Watson–Crick base paired DNA molecules. Inside the cell, however, the DNA often presents changes in its chemical structure, including modified bases, mismatches, or abasic sites. These chemical modifications can occur in the form of epigenetic marks via controlled action of the cellular machinery; or in the form of DNA lesions that jeopardize the normal functioning of the cell.

In this section, we review recent findings on the effects of epigenetics and DNA mismatches on DNA mechanics. When put together, these findings reveal that such chemical modifications have a more complex effect on DNA flexibility than previously thought. Methylation marks play a versatile role in DNA flexibility attending to the sequence context on which they occur and the mechanical deformation considered. On the other hand, DNA mismatches usually enhance DNA flexibility, but confer the DNA with exotic mechanical properties that remain to be deciphered. The emerging picture is that, in both cases, DNA flexibility might act as a potent signal for downstream events. Changes in DNA flexibility resulting from epigenetic marks might affect the compaction state of chromatin; whereas DNA defects might act as flexibility antennas for the recruitment of the repair machinery.

The impact of cytosine methylation on DNA physical properties

Epigenetic DNA marks play a myriad of roles in development (Smith and Meissner, 2013), aging (Unnikrishnan *et al.*, 2019), and the onset and progression of cancer (Esteller, 2008). The most common of these marks consists of the addition of a methyl group to the C5 carbon of cytosine, which typically occurs at CpG steps. The resulting methylcytosine is usually linked to gene silencing, but the molecular mechanism responsible for this is not completely understood (Cortini *et al.*, 2016). Notably, besides affecting the interaction of DNA with several proteins, cytosine methylation is suspected to orchestrate rearrangements of nucleosomes via changes in the mechanical properties of DNA (Dantas Machado, 2014; Cortini *et al.*, 2016).

Cytosine methylation is generally considered to reduce DNA flexibility Cortini *et al.* (2016), as supported by recent single-molecule cyclization experiments. Ngo *et al.* (2016) reported that upon methylation of one to eight (four in each strand) cytosines of an arbitrary DNA sequence, the looping time increases,

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Table 3. Effect of cytosine methylation on DNA physical properties

DNA physical property	Effect of cytosine methylation	References
DNA flexibility	Generally stiffens the DNA	Ngo *et al.* (2016)[a] Pérez *et al.* (2012); Portella *et al.* (2013)[b]
	Could increase the flexibility of CGIs	Pongor *et al.* (2017); Shon *et al.* (2019)[a] Marin-Gonzalez *et al.* (2019*a*)[b]
DNA melting	Versatile effect on DNA strand separation depending on the sequence	Severin *et al.* (2011)[a]
DNA structure	Affects DNA structure in a complex, sequence-dependent manner	Pérez *et al.* (2012); Rao *et al.* (2018)[b]
B–Z DNA transition	Facilitates B–Z transition under torsional stress	Lee *et al.* (2010*a*)[a]
DNA condensation	Promotes DNA condensation	Yoo *et al.* (2016); Kang *et al.* (2018); Yang *et al.* (2020)[a]

[a]Single-molecule experiments.
[b]Computer simulations.

which reflects a decrease in flexibility. This methylation-induced stiffening was well reproduced in supporting MD simulations, showing that methylcytosine dampened local fluctuations in the duplex. In addition, Ngo *et al.* (2016) combined OT and smFRET to show that methylation of the 601 Widom sequence resulted in mechanical destabilization of nucleosomes.

The idea that methylation stiffens the DNA is generally well accepted (Pérez *et al.*, 2012; Portella *et al.*, 2013; Cortini *et al.*, 2016). Yet, simulation (Marin-Gonzalez *et al.*, 2019*a*; Liebl and Zacharias, 2018) and experimental studies (Pongor *et al.*, 2017; Shon *et al.*, 2019) have indicated that, in some cases, methylation can soften the DNA. In particular, methylation could enhance the flexibility of CGIs (discussed in Section 'CpG islands are rigid DNA regions depleted of nucleosomes') (Marin-Gonzalez *et al.*, 2019*a*; Shon *et al.*, 2019; Pongor *et al.*, 2017). Dense methylation (hypermethylation) of CGIs is a potent gene silencing mechanism, and a hallmark of many cancer types (Esteller, 2008; Deaton and Bird, 2011). A possible role of DNA flexibility has been suggested in this process (Marin-Gonzalez *et al.*, 2019*a*; Shon *et al.*, 2019; Pongor *et al.*, 2017). In fact, CGIs appear rigid, a feature that might explain the nucleosome-binding deficient character of these sequences *in vivo* (see Section 'CpG islands are rigid DNA regions depleted of nucleosomes') (Pongor *et al.*, 2017; Shon *et al.*, 2019). However, upon hypermethylation, CGIs have been reported to become more flexible, with values of persistence length and stretch modulus closer to those of standard DNA (Pongor *et al.*, 2017; Marin-Gonzalez, 2019*a*; Shon *et al.*, 2019). This softening upon hypermethylation could increase the affinity of CGI to form nucleosomes, which may occlude the DNA to the transcription machinery, resulting in gene inactivation. Therefore, despite methylation is usually thought to stiffen the DNA, the full story might be more complex. Given the key biological function of CGIs, it would be of utmost interest to determine whether these sequences constitute an exception to the aforementioned rule.

Moreover, it is worth mentioning that cytosine methylation can have a substantial impact on DNA physical properties other than flexibility (see Table 3). For example, cytosine methylation affects force-induced DNA strand separation, as probed by single-molecule force spectroscopy measurements (Severin *et al.*, 2011). In this case, cytosine methylation plays a versatile role in DNA mechanics depending on the sequence-context: one methylated cytosine destabilized the duplex but three methylated cytosines resulted in larger mechanical stability. This versatility also appears to be present in sequence-dependent effects of cytosine methylation on DNA structure, as predicted from computer simulations (Pérez *et al.*, 2012; Dantas Machado *et al.*, 2014; Rao *et al.*, 2018). For example, in some sequence contexts, cytosine methylation would greatly affect the structure of the DNA, whereas in other sequences the structural effect of this epigenetic mark would be minimal (Rao *et al.*, 2018). In the extreme case of poly(dG–dC) sequences, single-molecule experiments showed that cytosine methylation can even facilitate the formation of non-canonical Z-DNA structures in the presence of torsional stress (Lee *et al.*, 2010*a*).

Finally, cytosine methylation has also been studied in the context of DNA condensation by polycations, the phenomenon by which highly positively charged ions mediate DNA compaction by stabilizing inter-helical interactions (Bloomfield, 1997). smFRET, MT, and MD simulations coincide that cytosine methylation enhances DNA condensation (Yoo *et al.*, 2016; Kang *et al.*, 2018; Yang *et al.*, 2020). Importantly, this effect appears consistent when using different condensing polycations (spermidine^{3+}, CoHex^{3+}, spermine^{4+}, and polylysine^{6+}), and DNA sequences.

Altogether, some general conclusions can be derived concerning the effect of cytosine methylation on DNA physical properties. Methylation usually reduces DNA flexibility and facilitates DNA condensation. Nevertheless, in many instances the role of cytosine methylation in DNA mechanics appears strongly dependent on the sequence context on which this epigenetic mark occurs. Therefore, methylation would add an additional layer of complexity to the sequence-dependent biophysical properties of DNA, instead of exerting a systematic, generalizable effect. Understanding cytosine methylation in the context of DNA mechanics would then require investigation on a case-by-case basis.

The effect of DNA mismatches on DNA mechanics

DNA defects, such as mismatches, can induce strongly distorted DNA conformations. Inside the cell, such DNA defects are localized and repaired by the coordinated action of DNA repair proteins with an impressive efficiency. However, the mechanisms by which some of these proteins rapidly identify single DNA imperfections in a huge genome are not completely understood. Remarkably, several DNA repair proteins spanning diverse repair pathways have been reported to interact with a sharply bent DNA (Roberts and Cheng, 1998; Natrajan *et al.*, 2003; Qi *et al.*, 2009; Chakraborty *et al.*, 2017; Craggs *et al.*, 2019; Paul *et al.*, 2019). A natural question is how (and to which extent) the deformability of damaged DNA impacts these repair processes and, in particular, the recognition

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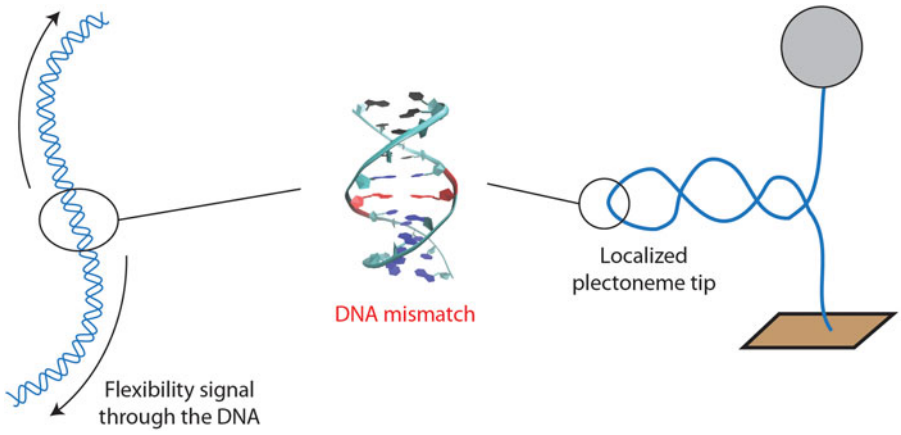


Fig. 3. Effects of DNA mismatches on DNA mechanical properties. A single mismatched base pair (center; PDB: 1ONM; Sanchez *et al.*, 2003) can propagate a mechanical signal through the DNA via an allosteric mechanism (left); and can pinpoint the position of a plectoneme tip (right).

of the defect (Krokan and Bjørås, 2013; Marteijn *et al.*, 2014; Kunkel and Erie, 2015). For example, do DNA defects spontaneously bend the duplex to recruit repair proteins or do these defects facilitate DNA bending once the protein is attached?

The answer to this question will greatly vary attending to the particular DNA defect and repair proteins involved. Here, we will discuss the effect of mismatches, or 'pairing' between non-complementary bases, which have recently received special attention in the context of DNA flexibility (Rossetti *et al.*, 2015; Jeong and Kim, 2019). Mismatches arise during DNA replication or DNA exposure to damaging agents, among other processes. Although these defects are generally associated with increased DNA bendability (see e.g. Vafabakhsh and Ha, 2012; Satange *et al.*, 2018), this might not always be the case, as suggested by recent studies (Rossetti *et al.*, 2015; Jeong and Kim, 2019). Using MD simulations and nuclear magnetic resonance experiments, Orozco, Gonzalez, and coworkers performed a systematic study of the effect of mismatches on DNA flexibility (Rossetti *et al.*, 2015). They observed that, in general, the magnitude of bending fluctuations was similar in mismatched duplexes and in control ones with correct base pairing, suggesting that mismatches do not enhance DNA bending flexibility. Nevertheless, DNA molecules containing mismatches were flexible at a local level, with frequent breathing events and distortions of the DNA grooves. The authors warn against simplistic interpretations and indicate that the aforementioned effects are largely dependent on the kind of mismatch considered. Nevertheless, their results suggest that, even though mismatches might not cause spontaneous DNA bending, local DNA distortions due to mismatches may aid proteins at bending the duplex.

Interestingly, Orozco, Gonzalez, and coworkers also observed that DNA distortions induced by defects not only occurred at the position of the mismatch, but also at remote locations of ~4 bp from the mismatch. This finding implies that mismatches might take advantage of a phenomenon known as DNA allostery (Kim *et al.*, 2013) to propagate distortions along the duplex. An independent, experimental observation of such long-range effects of mismatches has been recently reported in the context of single-molecule DNA cyclization (Jeong and Kim, 2019). Jeong *et al.* reported that, paradoxically, mismatches destabilize DNA loops, even though these defects favor the sharply bent DNA conformations that are required for loop formation. Such destabilization of loops was attributed to allostery effects, similar to the ones described in Rossetti *et al.* (2015), but reaching even longer distances of ~50 bp.

Altogether, the appealing hypothesis that mismatches propagate deformations along the DNA and, thus, act as 'flexibility antennas' for DNA repair proteins will require further research (see Fig. 3). Alternative mechanisms for the cellular machinery to identify mismatches should also be explored, such as the recently reported finding that mismatches can localize plectoneme tips (Ganji *et al.*, 2016; Dittmore *et al.*, 2017) (see Fig. 3). Note that these two mechanisms, DNA allostery and plectoneme localization, are not necessarily mutually exclusive. Finally, it remains to be explored whether these and other new phenomena arise from other kinds of DNA defects, such as oxo-guanines, photoproducts, or interstrand crosslinks.

Mechanical properties of dsRNA: unexpected differences with dsDNA

Single-molecule studies have also addressed the mechanical properties of dsRNA (Abels *et al.*, 2005; Herrero-Galán *et al.*, 2013; Lipfert *et al.*, 2014; Fu *et al.*, 2020). RNA is known to be predominantly single-stranded; however, dsRNA helices are also commonly found inside the cell and exert a myriad of biological functions. For example, dsRNA is the carrier of genetic information in some viruses and dsRNA helices are key elements of tertiary RNA structures, as formed e.g. by ribosomal RNA or t-RNAs (Carter *et al.*, 2000; Nissen *et al.*, 2001; Schimmel, 2018). The mechanical properties of dsRNA might play a role in some of these biological systems. For example, the dsRNA bending stiffness is expected to affect the energetics of genome compaction in dsRNA viruses (Zhang *et al.*, 2015; Buzón *et al.*, 2020). Moreover, the sequence-dependent structure and flexibility of dsRNA helices have been proposed to impact dsRNA:protein interactions or the folding of tertiary RNA structures (Perona and Hou, 2007; Yesselman *et al.*, 2019). Finally, a quantitative understanding of dsRNA mechanics could aid the future design of RNA nanostructures (Guo, 2010).

Initial studies on the dsRNA persistence length suggested that the mechanical properties of this molecule were qualitatively similar to dsDNA (Hagerman, 1997; Abels *et al.*, 2005). In a seminal study that combined MT and AFM, Abels *et al.* (2005) obtained a value of P_{RNA} ~62 nm, which is only 20% larger than P_{DNA}. This slightly larger rigidity of dsRNA can be easily rationalized on the basis of the thicker and more compact structure of the A-RNA helix compared to the B-DNA helix. In parallel, MD simulations indicated some differences in the dynamics of dsDNA and dsRNA at the microscopic level (Noy *et al.*, 2004). Namely,

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Table 4. Differences in the mechanical properties of dsDNA and dsRNA

Feature	dsDNA	References	dsRNA	References
Microscopic flexibility	Complex deformability, including e.g. allostery, polymorphic behavior	Kim *et al.* (2013)[a] Dans *et al.* (2014); Pasi *et al.* (2014)[b]	Simple deformability patterns	Noy *et al.* (2004); Beššeová *et al.* (2012)[b] Pérez *et al.* (2004)
Stretching stiffness	High ($S \sim 1200$ pN)	Smith *et al.* (1996); Wang *et al.* (1997)[a]	Low ($S \sim 400$ pN)	Herrero-Galán *et al.* (2013); Lipfert *et al.* (2014)[a]
Twist–stretch coupling	Negative: overwinds when stretched	Gore *et al.* (2006*a*); Lionnet *et al.* (2006)[a]	Positive: unwinds when stretched	Lipfert *et al.* (2014)[a] Liebl *et al.* (2015); Bao *et al.* (2017); Marin-Gonzalez *et al.* (2017)[b]
Plectoneme formation	Fast (ms) dynamics	Crut *et al.* (2007); Forth *et al.* (2008); Brutzer *et al.* (2010); van Loenhout *et al.* (2012)[a] Ott et al. (2020)[b]	Slow (s) dynamics	Lipfert *et al.* (2014)[a] Ott *et al.* (2020)[b]
Effects of multivalent (⩾2) cations	Increase bending flexibility; cause dsDNA condensation	Baumann *et al.* (1997); Wenner *et al.* (2002)[a]	Can decrease bending flexibility; dsRNA resists condensation	Fu *et al.* (2020)[a] Tolokh *et al.* (2014); Drozdetski *et al.* (2016)[b] Li *et al.* (2011); Katz *et al.* (2017)
Intrinsic bending	Occurs at A-tracts	Rivetti *et al.* (1998); Moreno-Herrero *et al.* (2006)[a]	Occurs at AU-tracts	Marin-Gonzalez *et al.* (2020*b*)[a]
Rigid motifs	A-tracts, CGI, ApT step	Shon *et al.* (2019); Marin-Gonzalez *et al.* (2020*a*) [a] Pasi *et al.* (2014)[b]	Homopurine regions: poly(rG:rC), poly(rA:rU) and poly(rA–rG)	Marin-Gonzalez *et al.* (2019*b*)[b]

[a]Single-molecule experiments.
[b]Computer simulations.

dsRNA showed simple deformability patterns that could be well described by few essential motions, whereas dsDNA was able to explore a wider range of conformations. Nonetheless, it was not clear whether these microscopic motions would translate into changes in the global flexibility of the molecules that could be measured in single-molecule experiments.

In the last few years, a number of single-molecule and simulation studies have revealed that the mechanical properties of dsDNA and dsRNA are more different than previously thought (Table 4). The emerging picture is that previous findings on dsDNA flexibility do not necessarily apply to dsRNA. Namely, the latter has its own mechanical identity. In the following, we discuss some of those studies. First, we discuss differences in the flexibility of DNA and RNA duplexes of arbitrary sequence under standard ionic conditions (Sections 'Stretching flexibility of dsDNA and dsRNA' and 'The opposite twist–stretch coupling of dsDNA and dsRNA'). We then comment on the opposite effect of certain multivalent ions on the mechanics of DNA and RNA duplexes (Section 'The different dynamics of plectoneme formation'). Finally, we discuss sequence effects on the structure (Section 'Opposite effects of complex ions on the mechanics of dsDNA and dsRNA') and the flexibility (Section 'Sequence determinants of intrinsic bending') of dsDNA and dsRNA.

Stretching flexibility of dsDNA and dsRNA

The first single-molecule study that reported qualitative differences in the mechanics of dsDNA and dsRNA was Herrero-Galán *et al.* (2013). The authors first performed AFM and MT experiments on dsDNA and dsRNA and found similar values of the persistence length to the ones previously published in Abels *et al.* (2005). Nonetheless, OT stretching experiments unveiled an important difference between the two nucleic acids: dsRNA was very soft to stretching deformations, around threefold more flexible compared to dsDNA (see Fig. 4*a*).

This different stretching flexibility of dsDNA and dsRNA has been reproduced in several experimental and computational studies (Chou *et al.*, 2014; Lipfert *et al.*, 2014; Bao *et al.*, 2017; Marin-Gonzalez *et al.*, 2017; Fu *et al.*, 2020). At the molecular level, the softer stretching response of dsRNA is commonly associated with the more open structure of the A-RNA helix compared to B-DNA (Chou *et al.*, 2014; Bao *et al.*, 2017; Marin-Gonzalez *et al.*, 2017). Note that this idea is similar to the one presented in Section 'Poly(dA–dT) and poly(dG:dC) adopt A-like DNA conformations', according to which DNA sequences with larger A-philicity would possess a softer stretching rigidity.

The opposite twist–stretch coupling of dsDNA and dsRNA

Measurements of the torsional response of dsDNA and dsRNA, as performed by Lipfert *et al.* revealed more striking differences in the mechanics of these duplexes (Lipfert *et al.*, 2014). Note that when a double-helix is straightened, two parallel strands reach a longer extension than they have when coiled around each other in a helical conformation. According to this picture, stretching a double-helix should lead to unwinding. However, it was known that dsDNA had the surprising ability of overwinding when stretched (Gore *et al.*, 2006*a*; Lionnet *et al.*, 2006) (see Fig. 4*b*). Using MT, Lipfert *et al.* measured changes in the dsRNA extension as a function of twist, and they found the completely opposite behavior: contrary to dsDNA, dsRNA unwinds upon stretching Lipfert *et al.* (2014).

The opposite twist–stretch coupling of dsDNA and dsRNA resulted in a great challenge for computational models (see e.g. the review by Kriegel *et al.*, 2017*a*). Several coarse-grain models,

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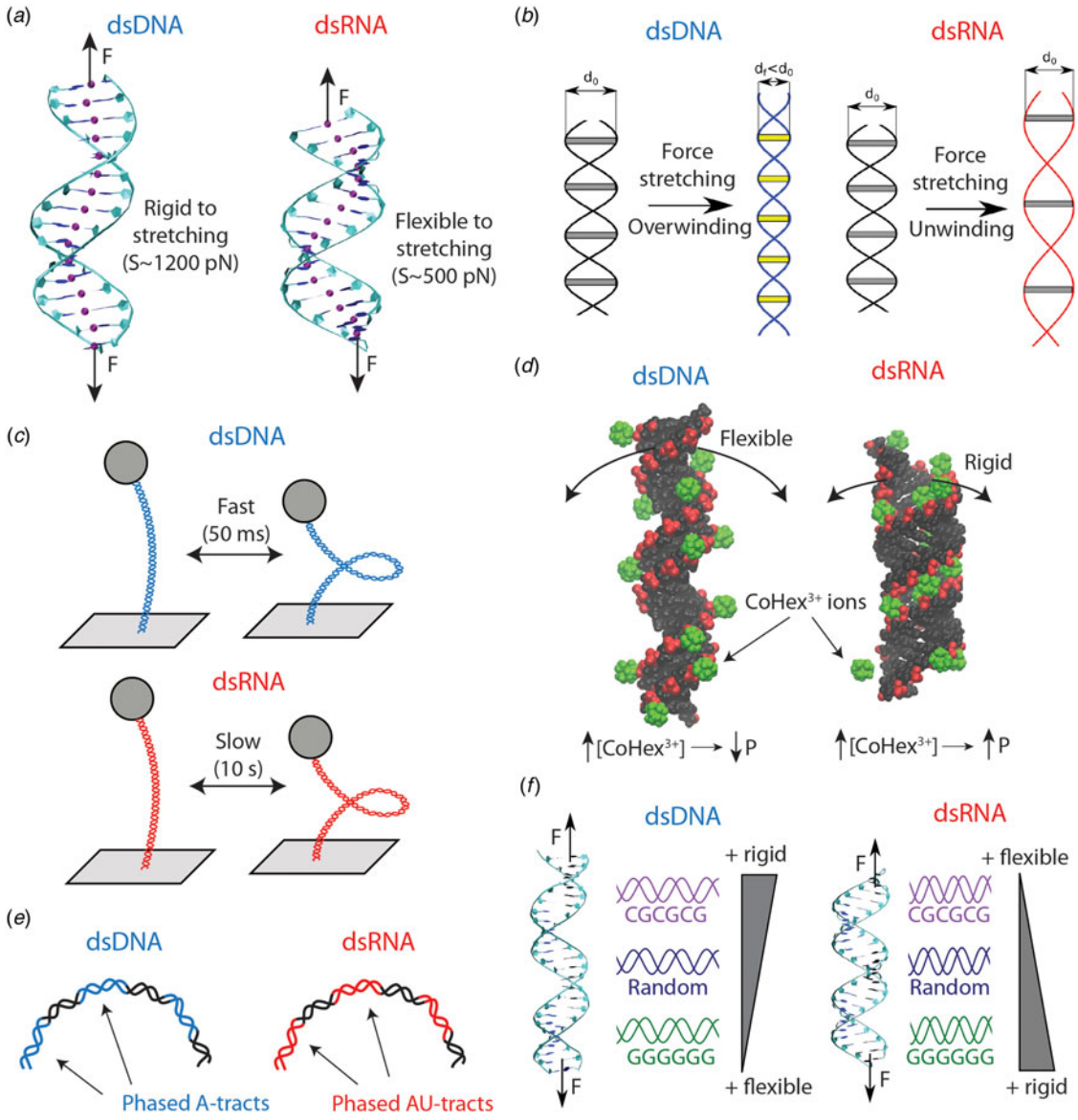


Fig. 4. Differences in the mechanical properties of dsDNA and dsRNA (see also Table 4). (*a*) dsRNA is around three times softer to stretching deformations than dsDNA. This difference can be explained on the basis of the more open structure of dsRNA, as evidenced from the base pair center chains of the duplexes (purple beads). (*b*) dsDNA overwinds when stretched, whereas dsRNA unwinds. The peculiar behavior of dsDNA can be rationalized from the shrinking of its radius upon elongation. On the contrary, the dsRNA radius is unchanged when the molecule is stretched. Adapted from Marin-Gonzalez *et al.* (2017). (*c*) When the duplexes are supercoiled at the threshold for plectoneme formation, dsDNA displays fast buckling dynamics (ms), whereas dsRNA undergoes slow (s) buckling transitions. This can be partly attributed to the larger persistence length of dsRNA; however, the precise mechanisms for this difference remain incompletely understood. (*d*) Increasing ionic concentrations result in larger dsDNA bending flexibility and a decrease in persistence length (*P*). However, some multivalent ions, such as $CoHex^{3+}$ or $spermine^{4+}$, can have the opposite effect on dsRNA and stiffen this duplex increasing *P*. This phenomenon can be understood from the shape of the grooves of the duplexes. For dsRNA, the ions can bind inside the major groove, but in the dsDNA case the ions bind mostly externally. Adapted from Drozdetski *et al.* (2016). (*e*) Sequence-induced bending in dsDNA and dsRNA occur, respectively, via A-tract and AU-tract (alternating adenines and uracil) sequences. (*f*) The nucleotide sequence impacts in different ways the global mechanical properties of dsDNA and dsRNA. Poly(dC–dG) motifs are rigid in dsDNA, but flexible in the dsRNA case; whereas poly(dG:dC) ones are flexible in dsDNA but rigid in dsRNA. Adapted from Marin-Gonzalez *et al.* (2019*b*).

with varying levels of detail, unsuccessfully attempted to reproduce the opposite twist–stretch coupling of dsDNA and dsRNA (Kriegel *et al.*, 2017*a*). For example, a base pair level model built from crystal structures of dsDNA and dsRNA concluded that both molecules would unwind when stretched (Chou *et al.*, 2014). The oxDNA/oxRNA models, which were originally designed to reproduce the thermodynamics of duplex formation (base pairing and stacking interactions), yielded the correct twist–stretch coupling for dsRNA, but failed for dsDNA (Matek *et al.*, 2015*a*, 2015*b*).

The shortfall of coarse-grain simulations suggested that, in order to reproduce the opposite twist–stretch coupling of dsDNA and dsRNA, fine details of the dynamics of the duplexes might need to be considered. Atomistic MD simulations met this demanding condition, as was elegantly demonstrated by Liebl *et al.* (2015). In that study, the authors performed atomistic MD simulations of a dsDNA and a dsRNA molecule of analogous sequence. An analysis of the MD trajectories revealed that the correlation between elongation and twisting motions were of opposite sign for both duplexes, in semi-quantitative agreement with

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the experiments. The authors further corroborated this finding by exerting a controlled torque on the nucleic acids and measuring the associated change in extension. Consistently, they found that a positive torque (overwinding) caused lengthening of dsDNA, but shortening of dsRNA; and unwinding resulted in the opposite behavior (shortening of dsDNA and lengthening of dsRNA). Liebl *et al.* elaborated a technical explanation for the opposite twist–stretch coupling of dsDNA and dsRNA based on a wealth of structural parameters of the duplexes. Another study also addressed the twist–stretch coupling of DNA and RNA duplexes and attributed their difference to the slide and inclination motions of the base pairs (Bao *et al.*, 2017).

Recently, an alternative mechanism for the twist–stretch coupling has been proposed, by directly computing the radii of the duplexes from MD simulations (Marin-Gonzalez *et al.*, 2017) (see Fig. 4*b*). DNA and RNA duplexes were stretched using a novel constant force protocol for MD simulations Liebl *et al.* (2015). It was found that, as the force increased, dsDNA overwound, while dsRNA unwound, in agreement with the aforementioned simulations and experiments. Importantly, because the molecules were left to equilibrate under stress, it was possible to measure how their average structure changed at each value of the external force. It was thus observed that the dsDNA radius decreases with force, whereas the dsRNA radius remains approximately constant. The force-induced shrinking of the DNA radius brings together the strands of the duplex, allowing it to overwind upon stretching (see Fig. 4*b*). Since this capability of reducing the radius was absent in dsRNA, this molecule could only unwind when stretched. The larger flexibility of the DNA sugar was suggested to be responsible for promoting the reduction of the dsDNA radius.

The different dynamics of plectoneme formation

The extensive investigation of Lipfert *et al.* on the twisting dynamics of dsDNA and dsRNA yielded yet another surprising result (Lipfert *et al.*, (2014). When these double-stranded nucleic acids are twisted above a certain threshold, they undergo a buckling transition and start forming plectonemes (see Fig. 4*c*). Lipfert *et al.* (2014) studied the dynamics of dsDNA and dsRNA when the twist of duplexes is constrained at the threshold value for the buckling transition. At that point, pre-buckling and post-buckling states are equally populated and the nucleic acids continuously transition between states. The authors found that such buckling dynamics were much slower in dsRNA compared to dsDNA, with a difference in dwell times of at least two orders of magnitude.

This issue has been recently addressed in an insightful simulation study. Ott *et al.* (2020) modeled a dsDNA and a dsRNA molecule using Brownian dynamics simulations and a simple WLC model that contemplates bending and twisting deformations. They found that the difference in persistence length of dsDNA and dsRNA, although small, can result in an order of magnitude change in the buckling dynamics. This approach thus partially explains the experimental findings. However, further theoretical efforts are needed to arrive at a quantitative description of the buckling dynamics of these nucleic acids (see the recent perspective by Lankaš, 2020). We believe that such description should take into account the cross-talk between twisting and bending deformations, that is, the fact that twisting can facilitate bending. This so-called twist–stretch coupling parameter has recently been estimated for dsDNA (Nomidis *et al.*, 2017), but a solid measurement is still lacking in the dsRNA case.

Opposite effects of complex ions on the mechanics of dsDNA and dsRNA

The effect of salt conditions on dsDNA mechanics has been extensively studied for several decades (Harrington, 1978; Ha and Thirumalai, 2003). However, recent studies are providing very interesting insights into this topic (Brunet *et al.*, 2015*b*; Kriegel *et al.*, 2017*b*; Guilbaud *et al.*, 2019). Nonetheless, in contrast to DNA, much less is known about the effect of salt on dsRNA flexibility. Initial single-molecule experiments reported a qualitatively similar decrease in P_{RNA} and P_{DNA} with increasing monovalent salt (Herrero-Galán *et al.*, 2013; Lipfert *et al.*, 2014). *A priori*, these findings could suggest a similar dependence of DNA and dsRNA flexibility on salt conditions. However, further single-molecule experiments and MD simulations have reported unexpected results when multivalent ions are present (Drozdetski *et al.*, 2016; Fu *et al.*, 2020) (see Fig. 4*d*). Motivated by the different condensation properties of dsDNA and dsRNA (Li *et al.*, 2011; Tolokh *et al.*, 2014), Onufriev and coworkers performed atomistic MD simulations to explore the role of the ion $CoHex^{3+}$ in P_{RNA} and P_{DNA} (Drozdetski *et al.*, 2016). The simulations showed that P_{DNA} decreases with increasing $CoHex^{3+}$ concentration, in accordance with naïve intuition and with previous experiments (Baumann *et al.*, 1997). However, unexpectedly, increasing amounts of $CoHex^{3+}$ resulted in an increase of P_{RNA}. This stiffening of dsRNA was consistent when alternative multivalent ions were used, such as $spermine^{4+}$ or a hypothetical Na^{3+}. Recently, the opposite effect of $CoHex^{3+}$ on the flexibility of dsDNA and dsRNA has been experimentally demonstrated in a comprehensive study that combines both MT experiments and MD simulations (Fu *et al.*, 2020). Altogether, these studies illustrate the limitations of polyelectrolyte models to describe the interaction of dsDNA and dsRNA with complex, multivalent ions such as $CoHex^{3+}$. In these cases, molecular details of the double helices, such as shape of their grooves, must be taken into account in order to explain the effect of these ions on the elasticity of the duplexes.

Sequence determinants of intrinsic bending

The phenomenon of sequence-induced DNA bending by A-tracts raises the question of whether a similar effect can occur in dsRNA. This question is more complicated than one would expect. For example, the few structures of naked RNA duplexes available from X-ray crystallography experiments often present artifacts (Šponer *et al.*, 2018). This issue constitutes an important drawback for experimental validation of dsRNA MD simulations via X-ray crystallography data (Šponer *et al.*, 2018). Testing MD against single-molecule experiments can therefore be a promising route for exploring sequence-dependent dsRNA features. By combining MD simulations and AFM experiments, it has been recently shown that alternating adenines and uracils – or AU-tracts – bend the RNA duplex (Marin-Gonzalez *et al.*, 2020*b*) (see Fig. 4*e*). AU-tracts were long known to possess a peculiar structure at a local base pair level (Dock-Bregeon *et al.*, 1989), but their effect on global dsRNA features had remained largely unexplored. The MD simulations from Marin-Gonzalez *et al.* (2020*b*) revealed that dsRNA molecules with AU-tracts were systematically more bent than sequences lacking this motif. Motivated by this finding, dsRNA molecules were fabricated with repetitions of AU-tracts spaced by 11 bp and these molecules were imaged using an AFM. The images revealed a bent character in these AU-tract molecules, which was quantified

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by a value of the persistence length as low as ∼30 nm, about half the standard value of dsRNA (∼60 nm). These findings argue against simplistic conceptions of dsRNA as a regular helix. Further investigation will be required to elucidate the molecular mechanisms and the possible biological consequences of this novel phenomenon.

Sequence-dependent mechanical properties

The aforementioned sequence-dependent dsRNA bending demonstrates that the nucleotide sequence can affect in a very distinct manner the structure of dsDNA and dsRNA. Namely, A-tracts induce an intrinsic bend in dsDNA, but not in dsRNA; and AU-tracts have the opposite effect: they bend dsRNA, but not dsDNA (see Fig. 4*e*). Therefore, it would be natural to expect that the nucleotide sequence would also impact the relative flexibility of dsDNA and dsRNA in different ways. A recent simulation study suggests that this might be the case (Marin-Gonzalez *et al.*, 2019*b*). Using MD simulations, Marin-Gonzalez *et al.* measured the mechanical response of several dsRNA sequences and compared the results with dsDNA analogs of the same sequence. dsRNA molecules were always more flexible to stretching deformations than dsDNA ones, regardless of the sequence. However, the effect of the nucleotide sequence on the stretching flexibility was completely different in the two nucleic acids. For example, the poly(rG:rC) RNA duplex is relatively rigid to stretching, but a poly(dG:dC) DNA duplex is highly flexible when compared with an arbitrary sequence (see Fig. 4*f*). On the contrary, the poly(rG–rC) dsRNA molecule is flexible to stretching, but the poly(dG–dC) dsDNA is rigid. The molecular mechanisms behind this difference are to be examined. Furthermore, the idea that sequence effects on dsDNA and dsRNA flexibility can be substantially different still awaits experimental validation.

Conclusions and future perspectives

In this review, we have revisited recent single-molecule experiments and MD simulations studies on DNA mechanical properties. These studies are collectively providing a comprehensive, molecular description of DNA mechanics by assessing how microscopic chemical features of the double-helix impact its physical properties. Importantly, as we deepen into such molecular characterization, a rich sequence-dependent conformational variability of the double-helix emerges, which is often overlooked by classical polymer approaches. For example, both experiments and simulations suggest that, besides few exceptions, the DNA persistence length is relatively insensitive to the nucleotide sequence (Geggier and Vologodskii, 2010; Mitchell *et al.*, 2017). Even distorted DNA duplexes containing a mismatch appear to have a standard bending flexibility (Rossetti *et al.*, 2015). On the contrary, local, strong deformations such as kinks, bubbles or Z-DNA structures are strongly sequence-dependent (Rich and Zhang, 2003; Olson and Zhurkin, 2011; Fogg *et al.*, 2012). It is thus tempting to conclude that sequence effects on DNA mechanics are amplified under large mechanical stress, that is, when the duplex is forced to adopt a structure that substantially differs from the canonical B-DNA helix.

Furthermore, the studies here reviewed are enabling a better understanding of the mechanical impact of cytosine methylation. Several lines of evidence indicate that cytosine methylation affects many DNA physical properties in a highly complex manner that often depends on the particular sequence context (see Table 3 and reference Cortini *et al.*, 2016). The emerging view is that, rather than exerting a systematic effect on DNA mechanics, methylated cytosine acts as a 'fifth nucleotide' that expands the 'physical code' imprinted in a given DNA sequence. Similar considerations might apply to DNA mismatches, whose effects on DNA mechanics appear to be strongly dependent on the specific kind of mismatch considered.

Finally, we have revisited recent findings on dsRNA mechanics, focusing on those studies that reveal unexpected observations in the mechanical properties of dsRNA when compared to its DNA counterpart. From a molecular perspective, these studies raise an interesting consideration. Namely, that the presence of an extra –OH group in the sugar and the substitution of thymine by uracil have enormous implications in the physical properties of nucleic acids.

Despite the substantial progress made in the last few years, a number of important aspects of DNA mechanics will require further study. In the following, we comment on some of those aspects and we briefly discuss how an improved characterization of DNA physical properties can potentially impact other areas of biology, biophysics, and nanotechnology.

Sequence-dependent DNA mechanics beyond the elastic regime

In the elastic regime, the sequence-dependent DNA deformability can be accurately described from the analysis of structural databases (Olson *et al.*, 1998), or from extensive atomistic MD simulations (Pasi *et al.*, 2014; Walther *et al.*, 2020). However, as mentioned above, the nucleotide sequence largely affects the energetics of highly distorted DNA conformations beyond the elastic regime, such as kinks or bubbles. We have outlined in a qualitative manner the main sequence determinants of such sharp DNA deformations (see Table 2). However, a quantitative characterization of DNA dynamics beyond the elastic regime is a challenge that will need to be addressed in future studies. In this respect, novel high-throughput assays based on next-generation sequencing, such as the recently developed loop-seq assay (Basu *et al.*, 2020, 2021), offer an attractive platform to interrogate such sequence-dependent DNA mechanical properties.

It is important to note that DNA kinks, bubbles, and other distorted conformations appear in a number of structures of DNA: protein complexes, including the nucleosome core (Dickerson *et al.*, 1998; Olson and Zhurkin, 2011). Therefore, a quantitative understanding of the energetics of these conformations might shed light on several biological questions. In the paradigmatic case of nucleosome stability, DNA kinks might be more determinant than the smooth bending flexibility (Zhurkin and Olson, 2013). Together with other sequence-dependent features, such as DNA shape (Rohs *et al.*, 2009), DNA kinks might greatly contribute to the wide sequence-dependent variability of nucleosome affinity reported in *in vitro* experiments (Onufriev and Schiessel, 2019).

The future challenges regarding the sequence-dependent DNA mechanics beyond the elastic regime can be summarized as:

- To quantitatively characterize the energetics of formation of highly distorted DNA conformations, most notably kinks and local denaturation events.
- To devise novel assays for systematically evaluating the sequence-dependent energetics of formation of highly distorted DNA conformations.

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- To evaluate under which circumstances the *in vivo* mechanical stress, as found e.g. in the nucleosome core, is sufficient for stabilizing the formation of those highly distorted DNA structures.
- To incorporate non-elastic effects into current coarse-grain models of DNA to improve theoretical descriptions of DNA mechanics.

Toward a better characterization of cytosine methylation and DNA mismatches in the context of DNA mechanics

As discussed in the text, cytosine methylation is usually correlated with an increased rigidity of the DNA. However, there is evidence pointing toward possible exceptions to this rule, most notably, certain sequences with high density of CpG steps (or CGIs). These (and potentially other kinds of) DNA sequences where methylation results in DNA softening clearly deserve more attention, both for experiments and simulations. The latter should aim to elucidate the molecular mechanisms behind methylation-induced changes in DNA flexibility, which still remain obscure.

In addition, future efforts are needed to better characterize the effect of cytosine methylation on DNA condensation by polycations. This question is particularly timely, in light of recent experiments and simulations that have suggested a liquid–liquid phase separation mechanism for DNA condensation (Kang *et al.*, 2018; Shakya and King, 2018). It is thus conceivable that cytosine methylation might enhance the phase separation behavior of DNA.

Understanding how DNA mismatches alter the DNA physical properties is another exciting task for the near future. For example, the hypothesis that mismatches promote allosteric effects in the DNA would benefit from more extensive experimental support at the single-molecule level. Another interesting idea to test experimentally would be whether different kinds of mismatches possess different mechanical footprints. Testing transductions (non-complementary pyrimidine:purine base pairing) against transversions (pyrimidine:pyrimidine or purine:purine base pairing) would be a promising starting point. Because the geometries of these kinds of mismatches are very different, it is expected they will possess different mechanical properties amenable to experimental observation.

Altogether, we devise the following challenges for future studies on cytosine methylation and DNA mismatches in the context of DNA mechanics:

- To further test experimentally the effect of cytosine methylation on the dynamics of CGIs and explore other potential exceptions to the rule that methylation reduces DNA flexibility.
- To further test the effect of cytosine methylation on the condensation and, potentially, liquid–liquid phase separation behavior of DNA.
- To provide additional experimental evidence on allosteric DNA effects induced by DNA mismatches.
- To explore the variability of mechanical effects among different kinds of mismatches.

DNA and RNA mechanics at the service of nanotechnology

Quoting Richard Feynman, 'what I cannot create, I do not understand'. True comprehension of natural processes is achieved when those processes can be customized to fulfill our necessities. In the last few decades, the field of DNA nanotechnology has provided an outstanding example of this philosophy. DNA nanostructures can be designed from molecular models that incorporate well-characterized biophysical properties of the DNA, including mechanical parameters such as the persistence length, the twist stiffness, or the twist–stretch coupling (Dietz *et al.*, 2009; Ouldridge *et al.*, 2010; Castro *et al.*, 2011; Maffeo and Aksimentiev, 2020). Nevertheless, when conceiving DNA as nanomaterial, considerations of sequence effects are often limited to base pairing and stacking interactions (Doye *et al.*, 2013). Only in few cases, additional sequence-dependent features of dsDNA, such as the B–Z transition or A-tract curvature, have been exploited to achieve novel functionalities of DNA nanodevices, such as molecular switches or curved DNA trajectories (Mao *et al.*, 1999; Iric *et al.*, 2018). Incorporating sequence-dependent biophysical properties, such as the ones delineated in Table 2, in the design of future DNA nanodevices could therefore expand the potential of DNA as nanotechnological material. Recent exciting developments in coarse-grain models of DNA mechanics hold great promise for this ambitious goal (Ouldridge *et al.*, 2010; Edens *et al.*, 2012; Freeman *et al.*, 2014; Chakraborty *et al.*, 2018; Maffeo and Aksimentiev, 2020).

In addition to DNA, the field of RNA nanotechnology is becoming increasingly popular. An improved quantitative comprehension of sequence-dependent dsRNA structure and flexibility will surely accompany a sustained and solid development of the RNA nanotechnology field (Guo, 2010; Jasinski *et al.*, 2017). Note that the rules that govern dsRNA mechanics and structure are different from those of dsDNA (see Table 4 and references therein). Namely, RNA possesses its own material properties, different from those of DNA, and might therefore offer new, unforeseen possibilities in terms of molecular design. Exploiting the unique biophysical properties of RNA for nanotechnological applications will be an exciting challenge for the years to come.

In conclusion, we foresee the following challenges for expanding the application of nucleic acids in nanotechnology:

- To exploit sequence-dependent structural and mechanical features in the design of DNA-based nanostructures.
- To advance our current knowledge on dsRNA mechanics and exploit these and other biophysical properties of RNA for improved design of RNA-based nanostructures.

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