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Dependence of Nucleosome Mechanical Stability on DNA Mismatches

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Abstract

The organization of nucleosomes into chromatin and their accessibility are shaped by local DNA mechanics. Conversely, nucleosome positions shape genetic variations, which may originate from mismatches during replication and chemical modification of DNA. To investigate how DNA mismatches affect the mechanical stability and the exposure of nucleosomal DNA, we used an optical trap combined with single-molecule FRET and a single-molecule FRET cyclization assay. We found that a single base-pair C-C mismatch enhances DNA bendability and nucleosome mechanical stability. The increase in force required for DNA unwrapping from the histone core is observed for single base-pair C-C mismatches placed at three tested positions: at the inner turn, at the outer turn, or at the junction of the inner and outer turn of the nucleosome. The results support a model where nucleosomal DNA accessibility is reduced by mismatches, potentially explaining the preferred accumulation of single nucleotide substitutions in the nucleosome core and serving as the source of genetic variation during evolution and cancer progression. Mechanical stability of the nucleosome is also dependent on the species as we find that yeast nucleosomes are mechanically less stable and more symmetrical in the outer turn unwrapping compared to Xenopus nucleosomes.

eLife assessment

This manuscript reports **important** data on the stability of nucleosomes. **Convincing** evidence obtained by single-molecule FRET experiments shows that DNA unwrapping is found to be slower when a single CC base pair mismatch is introduced at three different positions. The work is carefully conducted and described clearly, but the biological significance and implications of the findings on cellular DNA metabolism remain unclear.

Introduction

DNA base-base mismatches are generated by nucleotide misincorporation during DNA synthesis or by chemical modification such as hydrolytic deamination of cytosine [1]. They are also introduced intentionally in some genome editing approaches. DNA mismatches, if unrepaired, are sources of genetic variation such as single nucleotide polymorphisms and point mutations which can alter the cellular phenotype and cause dysfunction, diseases, and cancer [1,2]. DNA mismatches also alter the physical properties of DNA such as local flexibility and conformational heterogeneity [3-5].

In eukaryotes, DNA is packaged into a basic unit, the nucleosome, which consists of 147 base pairs (bp) of DNA wrapped around a histone octamer core [6-8]. In vivo, nucleosomes are regularly arranged along DNA like “beads on a string”, with short linker DNA separating the beads [6,7]. It has been commonly observed that the rate of genetic variation along the genome is correlated with nucleosome positions: [9-12] the substitution rate is higher while the indel (insertion and deletion) rate is lower nearer the center of a positioned nucleosome. One possible explanation for this correlation is that though mismatches may be generated randomly along the DNA, nucleosomes impose a barrier preventing the repair machinery from detecting and repairing the mismatch, thus leading to substitutions [13]. However, it is unknown how mismatches may affect nucleosome mechanical stability and nucleosomal DNA unwrapping, which may affect accessibility of the nucleosomal DNA to the repair machinery.

In an earlier work, we demonstrated a correlation between DNA flexibility and nucleosome stability under tension using the 601 nucleosome positioning sequence [14]. We showed that the 601 DNA around the histone core can unwrap asymmetrically under tension. One side of the outer DNA turn unwraps at a lower force and the other side unwraps at a higher force. The direction of asymmetry is controlled by the relative DNA flexibility of the two DNA halves flanking the dyad. Unwrapping force is lower for the nucleosomal DNA side with lower flexibility and vice versa. In addition, cytosine modifications that make DNA more flexible made the nucleosome mechanically more stable and vice versa[15].

Here, we examined the effect of a DNA mismatch on DNA flexibility and nucleosome unwrapping dynamics. We used a single molecule DNA cyclization assay to examine the flexibility of DNA containing a mismatch, and a fluorescence-force spectroscopy method to study the effect of mismatch on nucleosome unwrapping dynamics. Similarly, we examined the mechanical properties of nucleosomal DNA assembled using yeast and Xenopus histones.

Results

Monitoring nucleosome unwrapping by fluorescence-force spectroscopy

To measure conformational dynamics of the nucleosome in response to external force we used a single-molecule assay that combines fluorescence resonance energy transfer (FRET) with optical tweezers [16-18]. This assay allows us to use FRET to probe local conformational changes of the nucleosome caused by tension applied by optical tweezers through the two ends of the nucleosomal DNA.

The nucleosome was reconstituted using the nucleosome positioning sequence 601, with or without a C-C mismatch. We designed three DNA constructs 601-R18, 601-R39, and 601-R56 with the mismatches at R18, R39, and R56 positions situated in the middle of the outer turn, at the junction between the outer turn and inner turn, and in the middle of the inner turn, respectively (**Fig. 1**). Because the distance between the mismatch positions (17, 21 and 38 bp) are not in multiples of 5 bp, they reside in different positions within their own super-helical turn. And the mismatches are at positions where the major groove face toward (R56) or away from (R18, R39) the histone core (R56) so they do not all share the same specific contacts with the histone octamer. Two fluorophores – Cy3 (FRET donor) and Cy5 (FRET acceptor) - were placed in appropriate positions to report on the unwrapping of various sections of nucleosomal DNA through reduction in FRET (**Figure 1** and Supplemental Figure S1). The two strands of the DNA construct were separately created by ligation of the component strands (Supplementary Fig. S1) to ensure that the resulting DNA does not contain a nick. The double-stranded construct was then formed by slowly annealing the two purified ligated strands over 3-4 hours. All four DNA constructs (601, 601-R18, 601-R39, and 601-R56) yielded nucleosomes with the same electrophoretic mobility and single molecule FRET value, indicating that the nucleosomes are homogeneously positioned for all four constructs (Supplementary Fig. S1).

In the fluorescence-force spectroscopy assay, a nucleosome was anchored to a polymer-passivated glass surface via biotin-neutravidin linkage on one end of the nucleosomal DNA. The other end of the nucleosomal DNA was attached to a bead held in an optical trap via a λ-DNA tether (**Fig. 1A**). As previously described [14], we attached a pair of donor and acceptor fluorophores to the DNA to probe the unwrapping of nucleosomal DNA. To probe the unwrapping of the outer DNA turn, we constructed DNA with a labeling scheme called ED1 (end-dyad 1) in which the donor is incorporated on the 68th nucleotide from the 5' end of the top strand (I68) and the acceptor is attached to the 7th nucleotide from the 5' end of the bottom strand (J7) (**Fig. 1A**). Upon nucleosome formation, the ED1 probe displayed high FRET due to proximity between the donor and the acceptor. We applied tension to the nucleosomal DNA by moving the piezo stage to which the glass surface attached at a constant speed of 455 nm/s while a focused laser (532 nm) follows the molecule to monitor fluorescence signals. The force was increased from a low value (typically between 0.4 – 1.0 pN) to a predetermined higher value and then returned to the low value by moving the stage in the opposite direction at the same speed (**Fig. 1**). We observed a gradual decrease in FRET - corresponding to an increase in the Cy3-Cy5 distance - as the force increases. Upon further increase in force, we observed rapid fluctuations in FRET, followed by a sharp decrease in FRET (**Fig. 1**), consistent with our previous studies [14,15] and a more recent study [19] utilizing high resolution optical tweezers with simultaneous smFRET detection. Upon relaxation through gradual decrease in force, the nucleosome reformed as reported via recovery of high FRET but at a lower force than the force at which unwrapping occurred, demonstrating mechanical hysteresis.

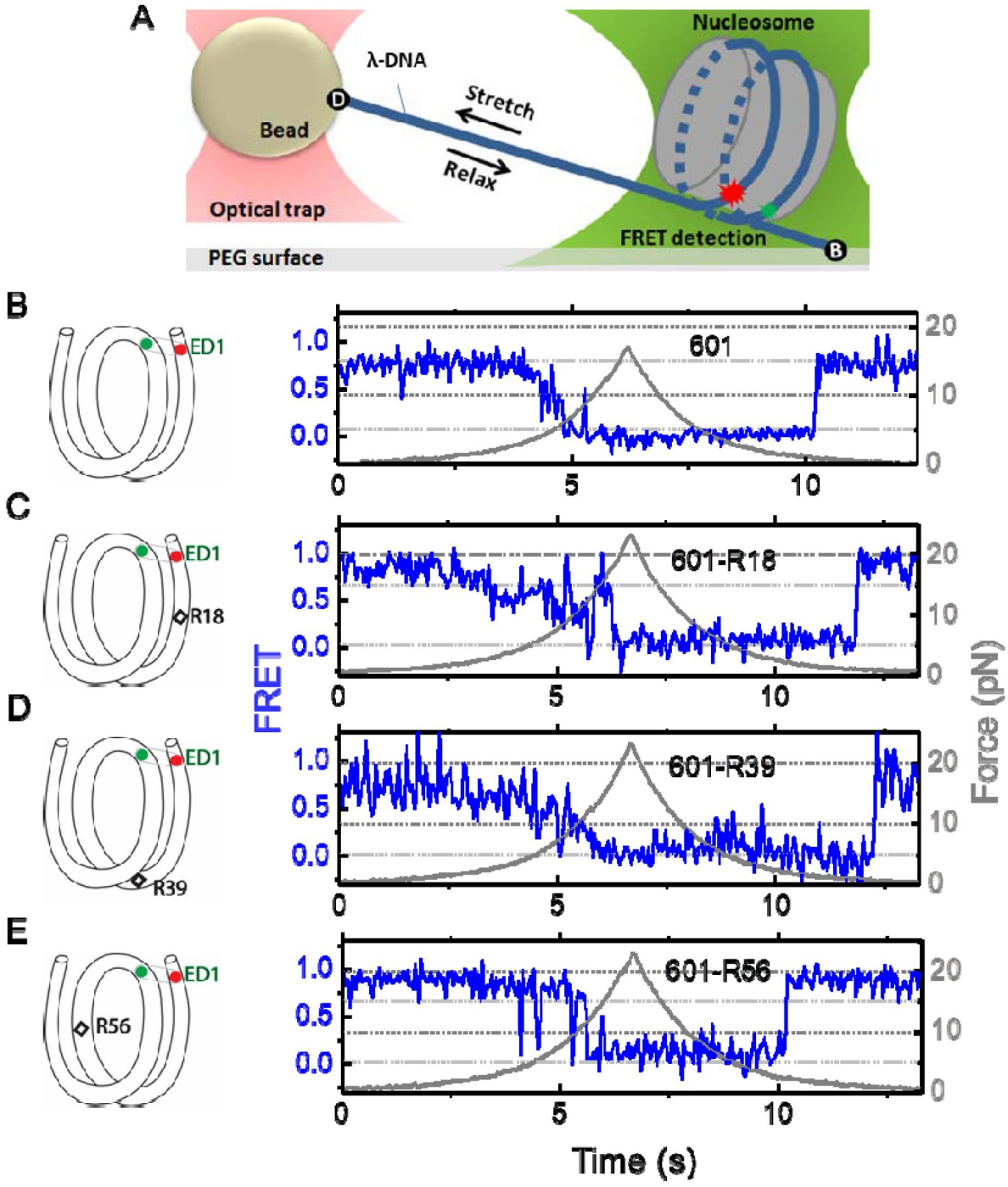

Figure 1

Nucleosome unwrapping measurement.

(**A**) Experimental scheme. The red and green stars represent labelled Cy5 and Cy3 fluorophores. (**B, C, D, E**): Representative stretching traces of the outer turn (ED1) for nucleosomes reconstituted from the 601 sequence (**B**) and from the 601 sequence with containing a mismatch at different positions: on the outer turn (**C**), at the junction of the outer turn and inner turn (**D**) and at the inner turn (**E**). The red and green dots on the DNA bends represent labelled Cy5 and Cy3 fluorophores. The elongated circles enclosing red and green dots represent the ED labeling position. The black diamonds on the DNA bends represent the mismatch position with R18 and R39 on minor grooves and R56 on a major groove.

History-dependent mechanical stability of mismatch-containing nucleosomes

Electrophoretic mobility shift analysis and zero-force FRET values did not show a noticeable difference between unmodified nucleosomes and mismatch-containing nucleosomes (Supplementary Fig. S1). However, under perturbation by force, although unmodified nucleosomes showed the same behavior between stretching cycles [14], mismatch-containing nucleosomes showed different behaviors between stretching cycles (**Fig. 2**). The ED1 side of the mismatch containing nucleosomes unwrapped at lower forces for the first few cycles and then at higher forces for subsequent cycles. After relaxation, we observed a general trend of an increase in unwrapping force in the subsequent stretching cycles. One possible explanation for this observation is the re-positioning of the nucleosome such that the mismatch moves toward the dyad, bringing the ED1 probe toward the interior of the nucleosome, as predicted by a previous theoretical model [20]. According to this model, the nucleosome position is weakly affected by the presence of a flexible lesion on the DNA, but under perturbation by other cellular components which either stiffen the DNA overall or weaken histone binding, the lesion can be made to have a strong preference for the dyad position. In our experiments, applied tension during stretching may act as perturbation which weakens nucleosome binding. When the probes move closer to the dyad in the subsequent stretching cycles, more base pairs of DNA would need to be unwrapped for FRET to decrease, potentially explaining the observed increase in unwrapping force. However, since the FRET values in our DNA construct are not sensitive to the nucleosome position, further experiments with one fluorophore positioned on the histones and one fluorophore positioned on the nucleosomal DNA will be required to test this hypothesis.

DNA C-C mismatch enhances nucleosome mechanical stability

We compared the FRET versus force curves for constructs containing one C-C mismatch each at three different locations: R18, R39 and R56. We observed similar stretching patterns for the mismatch-containing nucleosomes (601-R18, 601-R39, 601-R56) to that of the 601 nucleosomes. However, the force range where FRET reduced gradually accompanied by fluctuations was wider and extended to higher force for mismatch-containing nucleosomes (**Fig. 1 C-E**). Because we observed increases in unwrapping forces for the second stretching cycle and beyond for mismatch-containing nucleosomes, we only used the first stretching cycle for comparing unwrapping forces between constructs. The averaged FRET vs. force pattern for 601-R39 showed an increase in unwrapping force for the mismatch-containing nucleosomes (**Fig. 3A**). The increase in unwrapping force for all three mismatch containing constructs indicates that local flexibility of either the inner turn or the outer turn regulates nucleosome unwrapping (**Fig. 4**). Next, we probed unwrapping of the nucleosome on the side that does not contain the mismatch for the construct containing a mismatch at the R39 position. In this configuration named ED2 (end-dyad 2), the donor was placed on the inner DNA turn close to the dyad (J58) which is similar to the ED1 construct, and the acceptor was incorporated to the opposite ends (I9) (**Fig. 3B**). Stretching curves of ED2 nucleosomes formed on the 601 sequence yielded higher unwrapping force compared to the ED1 side as reported previously [14]. The mismatch construct yielded nearly the same unwrapping pattern as the 601 nucleosome, suggesting the change in local flexibility induced by the mismatch has a strengthening effect against unwrapping only for the side containing the mismatch (the ED1 side).

DNA C-C mismatch enhances DNA bendability

A single DNA mismatch can cause DNA to deviate from the B-form conformation [4,5] and increase DNA flexibility [3]. A previous study using a DNA buckling assay suggested that C-C is one of the most flexible mismatches [3]. Therefore, we chose C-C as a representative mismatch to investigate its effect on nucleosome stability. We hypothesized that the stabilization of the nucleosome forming on mismatch containing DNA sequences is caused by its increase in DNA

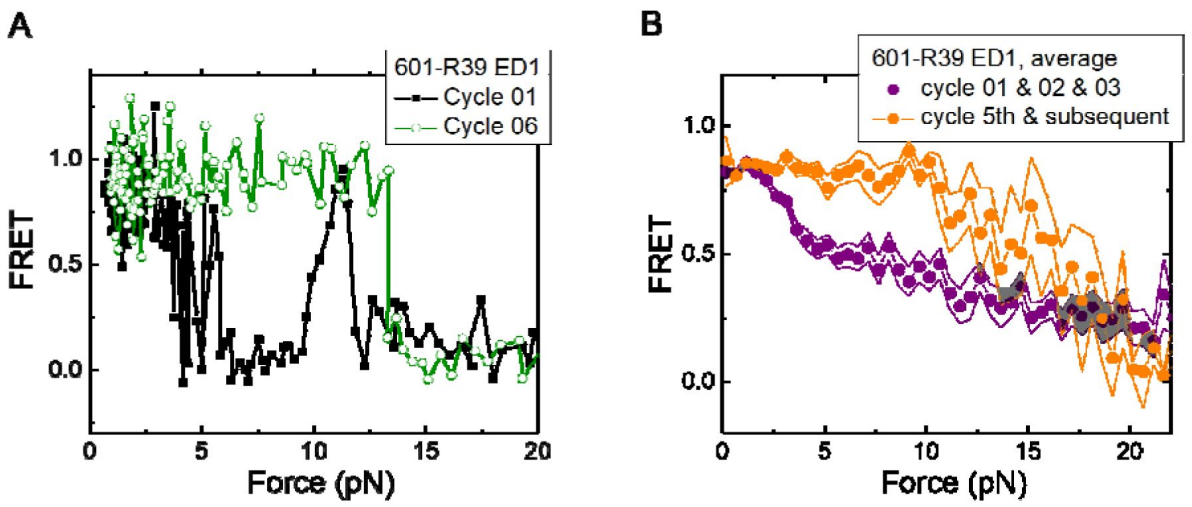

Figure 2

Unwrapping force of mismatch-containing nucleosomes is higher for subsequent stretching cycles.

(A) Representative single-molecule stretching traces at two stretching cycles from the sample molecule, probe by the ED1 FRET pair. (**B**) Averaging FRET vs. Force for many molecules at the first three stretching cycles (purple) and the subsequent stretching cycles (orange). Histone proteins were expressed in xenopus. The error bars represent S.D. of n = 25 and 11 traces for the first 3 stretching cycles (purple) and for the cycle 5th and the subsequent stretching cycles (orange), respectively.

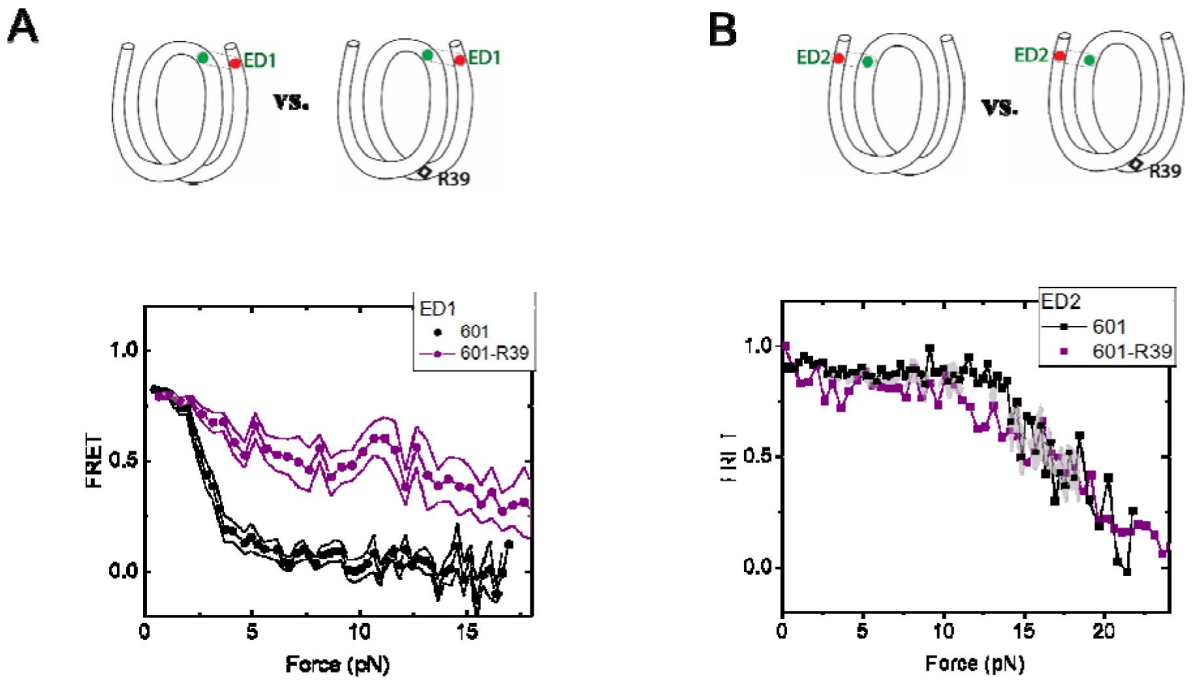

Figure 3

Enhancement of nucleosome mechanical stability by DNA mismatch.

Average of FRET vs. Force for ED1 probe (**A**) and ED2 probe (**B**) for the 601 nucleosome (black) and for the first stretching cycle of the mismatch containing nucleosome 601-R39 (purple). Histone proteins were expressed in xenopus. The error bars represent S.D. of n = 25 and 7 for the ED1 probe of the 601 and 601-R39 nucleosomes (**A**) and n = 20 and 39 for the ED2 probe of the 601 and 601-R39 nucleosomes (**B**), respectively.

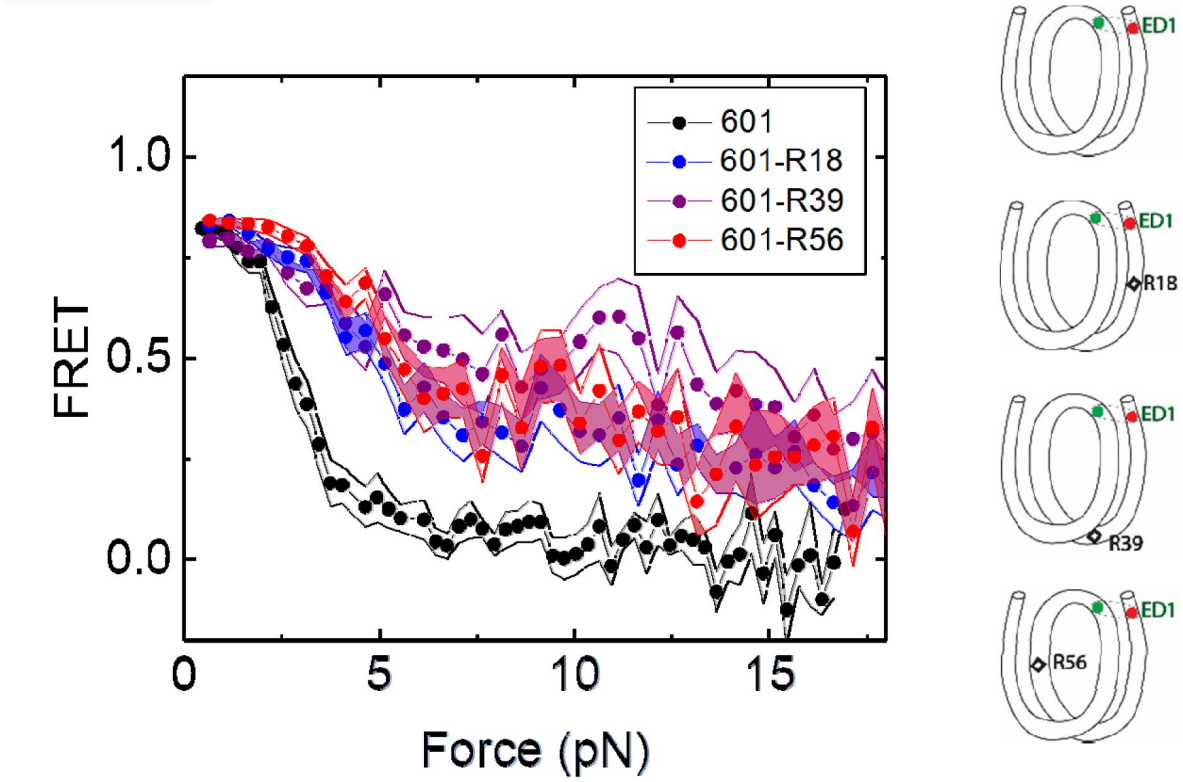

Figure 4

Mismatch position-dependence of nucleosome unwrapping.

Average of FRET vs. Force for ED1 probe for the 601 nucleosome (black) and the mismatch-containing nucleosome 601-R39 (purple), 601-R18 (blue) and 601-R56 (red). Histone proteins were expressed in xenopus. The error bars represent S.D. of n = 25, 11, 7 and 10 for the 601, 601-R18, 601-R39 and 601-R56 nucleosomes, respectively.

bendability. Therefore, we used a single molecule DNA cyclization assay [21] to probe the change in apparent bendability of the right half (RH) of the 601 sequence upon introducing the C-C mismatch. In this assay (**Fig. 5**), DNA fragments with two 10 nt long 5' overhangs were immobilized on a microscope slide. A FRET pair (Cy3 and Cy5) was incorporated at the 5' ends of the overhangs that are complementary to each other, allowing us to detect high FRET when the two overhangs anneal with each other forming a circle. We used smFRET to quantify the fraction of looped molecules versus time after the high salt buffer is introduced in the chamber. The rate of loop formation was used as a measure of apparent DNA flexibility influenced by a mismatch [22,23]. The faster the looping occurs, the more flexible the DNA is.

We measured the looping time of 4 DNA constructs corresponding to the right half of the 601 sequence (601-RH) with the addition of a C-C mismatch at the R18, R39 and R56 locations (601-R18-RH, 601-R39-RH and 601-R56-RH). As expected, we observed a dramatic decrease in looping time of the construct containing a mismatch (**Fig. 5B**). Adding a C-C mismatch reduced the looping time from 57 min to 32 min (601-R18-RH), 9 min (601-R39-RH), and 32 min (601-R56-RH). The reduction in apparent looping time was larger with the mismatch placing at the center (601-R39-RH) than toward the side of the RH fragment (601-R18-RH,601-R56-RH) likely because the looping measurement is more sensitive to the change in flexibility at the center of the DNA fragment.

The cyclizability of surface-tethered DNA constructs was shown to possess an oscillatory dependence on the distance of the biotin tether from the end of the molecule [24]. For example, moving the location of the biotin tether by half the helical repeat (~ 5 bp) can lead to a large change in cyclization rate [24]. We therefore performed control experiments to test the possibility that the observed higher cyclization rates of constructs with mismatches, as shown in **Fig. 5B-C**, is an artifact specific to the biotin tether location used. We created two additional constructs, 601-RH-16bp and 601-R18-RH-16bp, which are identical to the 601-RH and 601-R18-RH constructs, respectively, except that the location of the biotin tether was moved to a thymine base that lies 16 nucleotides further towards the center of the molecule. We chose 16 nucleotides because it is about 1.5 times the helical repeat, and thus cyclization rates should be maximally different when compared to the original 601-RH and 601-R18 constructs. Further, there was a thymine base present there to which the biotin could be conveniently attached. Side-by-side, we re-prepared the original 601-RH and 601-R18-RH constructs. We found that the overall looping rates of both the 601-RH-16bp and 601-R18-RH-16bp constructs were higher than for the 601-RH and 601-R18-RH constructs, indicating that moving the biotin tether towards the center of the molecule increases looping rate (**Fig. 5C**). However, the 601-R18-RH-16bp construct, which contains a mismatch, still looped faster than the 601-RH-16bp construct without a mismatch (**Fig. 5C**). We thus conclude that the presence of the C-C mismatch makes the construct loop faster, and that this is not an artifact specific to the biotin tether location.

Effects of other mismatches on DNA bendability

There are eight different types of mismatches made from canonical DNA bases: A-A, T-T, C-C, G-G, G-A, C-A, C-T and G-T mismatches. We performed single molecule looping experiments of DNA containing a single mismatch introduced near the middle of the looping construct and determined the looping times for all eight constructs (**Fig. 6**). See Supplementary Materials for their sequences. We observed significant reduction in looping time compared to the intact DNA control (no mismatch) with the exception of G-containing mismatches (G-G, G-T and G-A mismatches). The C-containing mismatches (C-C, C-A and C-T) showed the largest reduction in looping times, suggesting that they make DNA most bendable. We also compared our looping times with the published measure of DNA bendability for the corresponding mismatched DNA where they quantified DNA buckling via FRET (E_{FRET} in **Fig. 6**)[3]. The two measures generally agree. However, we found sizable deviations from a linear relation for T-T and G-T mismatches. It is possible that the deviations may arise from sequence contexts, but we cannot exclude the possibility that the two assays measure slightly different aspects of DNA mechanics. Although

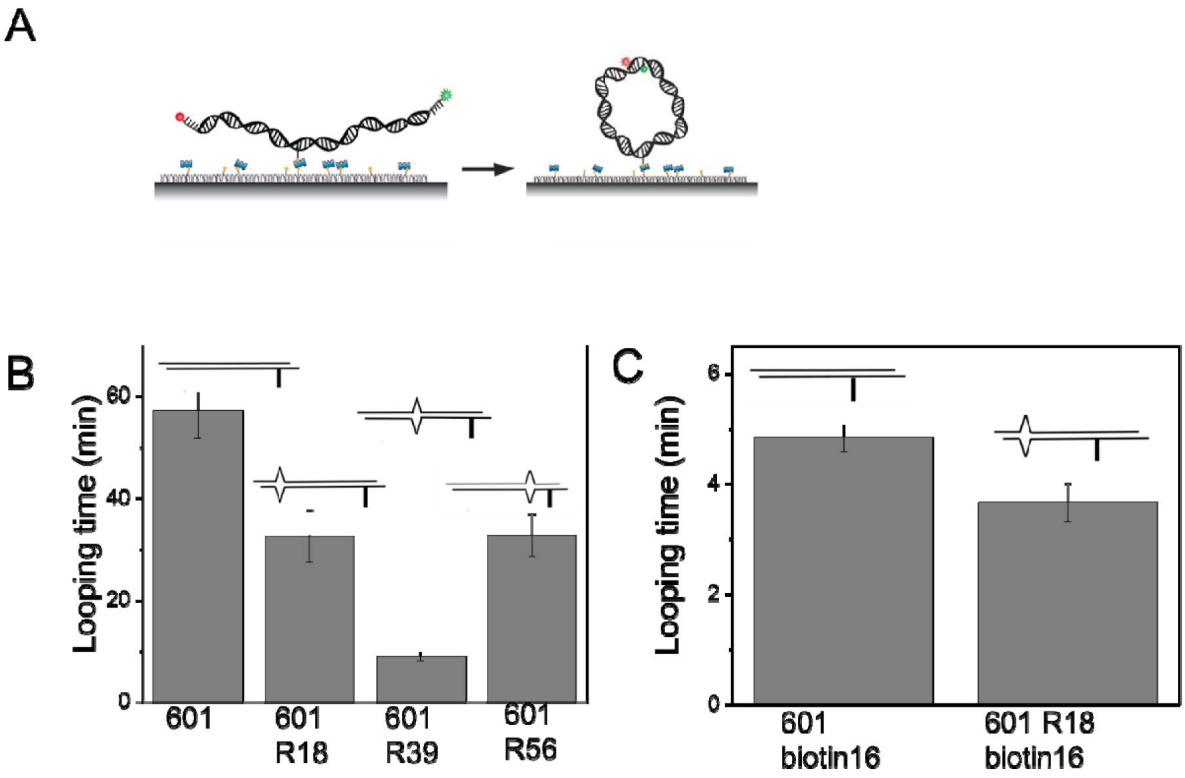

Figure 5

C-C mismatch enhances DNA flexibility.

(**A**): Single molecule cyclization assay: The DNA construct with 10-nucleotide complementary sticky ends is immobilized on a PEG passivated imaging chamber. DNA looping is induced using the imaging buffer containing 1M NaCl followed by time course TIRF imaging. To calculate the looping time, the fraction of looped molecules (high FRET) as a function of time is fitted to an exponential function. (**B**, **C**) Fitted looping time for the right half of the 601 construct without and with mismatches (**B**) and with the biotin position being moved by 16 nt (**C**). Error bars represented the S.E.M with n = 3 technical replicates.

performing fluorescence-force spectroscopy for non-C-C mismatches is beyond the scope of the current work, a testable prediction is that T-T and A-A mismatches as well as C-containing mismatches make a nucleosome mechanically more stable.

Yeast nucleosomes are less stable and more symmetrical than Xenopus nucleosomes in outer turn unwrapping

Next, we sought to examine how the source of histone proteins affects nucleosome stability. We reconstituted the 601 DNA construct with histone octamers of Xenopus and budding yeast. Note that all the data presented thus far on the effect of mismatches were obtained using Xenopus histones. Outer turn FRET probes on both sides ED1 and ED2 displayed slightly lower zero-force FRET values for yeast nucleosomes compared to Xenopus nucleosomes (**Fig. 7**), indicating that the DNA entry/exit may be more loosely bound on histone core for yeast nucleosomes. In contrast, the inner turn FRET probe showed similar zero-force FRET values for yeast and Xenopus nucleosomes. With pulling force applied, the stretching pattern for ED1 is similar for both nucleosomes while the strong side probe ED2 showed lower mechanical stability for yeast histones, with 40% of the molecules having unwrapping force of lower than 5 pN and the other 60% of the molecules being unwrapped by a force between 5-15 pN (**Fig. 7A-B**). The inner turn probe showed a stepwise unwrapping pattern with initial FRET reduction at less than 5 pN followed by stable FRET and a final unwrapping at a force higher than 20 pN (**Fig. 7C**). These observations for both outer turn and inner turn probes suggested that nucleosomes made with yeast histones are mechanically less stable, and unwrap less asymmetrically than nucleosomes made with Xenopus histones.

Discussion

Using the looping time of single molecule DNA cyclization as a measure of DNA bendability, we showed that a DNA mismatch can increase DNA bendability. Our results for the selected mismatches are consistent with previous studies on the effect of a mismatch on DNA conformational dynamics using other methods such as NMR [4] and the DNA Euler buckling assay [3]. A possible explanation for the enhancement of DNA flexibility is the existence of a kink at the mismatch position on the DNA.

We observed the enhancement of mechanical stability of nucleosomes reconstituted from mismatch-containing DNA constructs. A defect making the system more stable may appear counterintuitive but given that the same mismatch can make DNA more flexible, our findings are in broad agreements with previous studies that showed positive correlation between DNA flexibility and nucleosome mechanical stability when DNA sequences or cytosine methylation was altered [14,15].

The 601 positioning sequence has TA-rich side that has four TA dinucleotides spaced with 10 bp periodicity and is more flexible than the TA-poor side [14]. The 601 nucleosome is more stable on the TA-rich side [14,19,25], which we attributed to the ease with which the more bendable DNA stays sharply bent around the histone core even under unwrapping force. Here, we introduced a mismatch to the TA-poor side with the aim of achieving a large contrast in the background of rigid DNA. Indeed, the mismatch induced a ~7-fold increase in the rate of DNA cyclization of the TA-poor side. This increase in DNA flexibility matches the ~7 fold larger cyclization rate of the TA-rich side compared to the TA-poor side [14], suggesting that a single mismatch in the TA-poor side can symmetrize DNA flexibility of the 601 nucleosome. However, unlike flexibility symmetry achieved by TA repeats where which side unwraps at low forces became stochastic [14], when the flexibility symmetry was obtained via a mismatch in the TA-poor side, the TA-rich side remained mechanically stable, unwrapping at only high forces. This difference suggests that although the

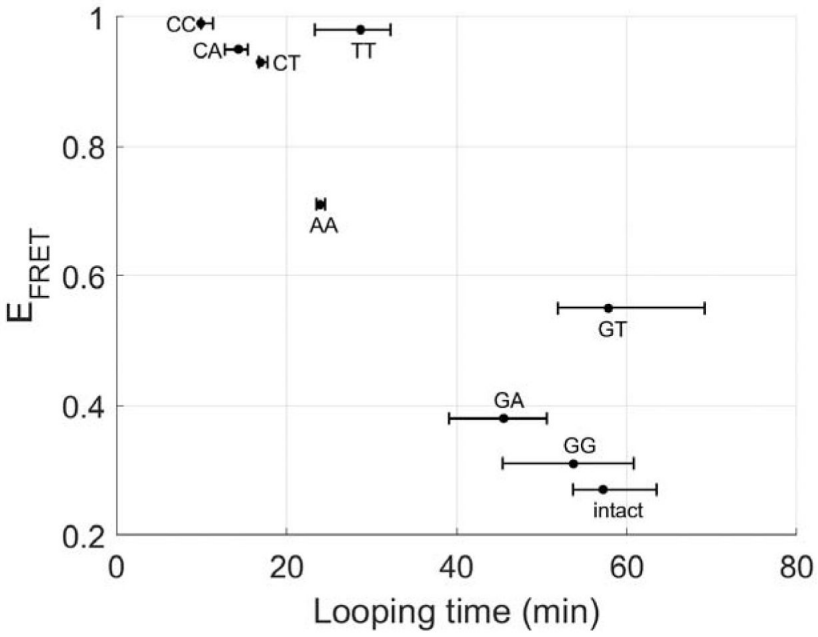

Figure 6

DNA flexibility enhancement is dependent on mismatch type.

Looping times for DNA containing a single mismatch (one of eight types each) and an intact DNA without a mismatch. Also shown are ensemble FRET efficiencies (E_{FRET}) from Ref. [3] as a measure of DNA buckling for the same type of mismatch.

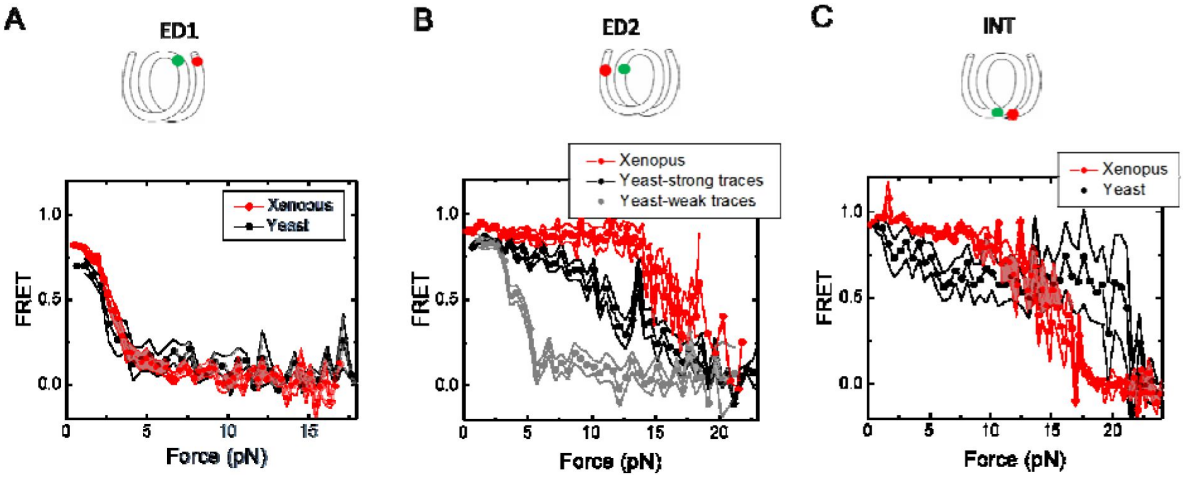

Figure 7

Unwrapping of yeast vs. xenopus reconstituted nucleosomes:

Average of FRET vs. Force for nucleosomes reconstituted from xenopus (red) vs yeast (black and gray) histone proteins with DNA labeled by outer turn probes ED1 (**A**), ED2 (**B**) and inner turn probe INT (**C**). The error bars represent S.D. of n = 17 (Xenopus) and 5 (Yeast) nucleosomes with the ED1 probe (**A**), n = 20 (Xenopus), 6 (Yeast – strong) and 4 (Yeast-weak) nucleosomes with the ED2 probe (**B**), and n = 22 (Xenopus) and 6 (Yeast) nucleosomes with the INT probe (**C**), respectively.

apparent flexibility is similar between DNA containing a mismatch vs a flexible sequence element, the mismatch does not have a global effect on the coordination of unwrapping of the two DNA ends.

The enhanced nucleosome mechanical stability we observed suggests that a mismatch will reduce nucleosomal DNA accessibility, providing a mechanism to potentially explain the accumulation of substitutions near the center of positioned nucleosomes[9-12] which may be the source for genetic variation during evolution and cancer progression. The reduction in nucleosomal DNA accessibility would hinder the activity of the DNA mismatch repair machinery on nucleosomal DNA. An unrepaired mismatch leads to a point mutation. In fact, previous observations showed that the frequency of single nucleotide polymorphism is higher near the nucleosome dyad [10]. The higher frequency of substitutions in the nucleosomal DNA may be attributed to the difficulty of accessing the extra-stable nucleosomes. More orthogonal experimental approaches are needed to test this model. We chose the C-C mismatch for this work because a previous study showed that the C-C mismatch is one of the most flexible mismatches [3]. If indeed more flexible elements in the DNA make a nucleosome mechanically stronger, as shown here for the C-C mismatch and previously for different sequences and cytosine modifications [14,15], we can predict that other mismatches, DNA lesions and alternative DNA structures such as DNA single strand damages, bulky adducts and R-loops [26] that alter DNA local flexibility would also change nucleosome mechanical stability accordingly. Future studies are needed to test this prediction.

We also tested if histones from different species and different histone variants can affect nucleosome stability under tension. We observed a slightly lower zero-force FRET value for both sides with ED1 and ED2 for yeast nucleosomes compared to Xenopus nucleosomes. Under tension, we found that the outer turn of yeast nucleosomes could be unwrapped at a lower force than Xenopus nucleosomes, and that the unwrapping pattern is less asymmetric for ~40% of nucleosomes formed on the 601 sequence. Therefore, how DNA mechanics is translated to nucleosome mechanics may be dependent on species-specific differences in histone sequence and post-translation modifications, which need to be examined in future studies.

While we are able to demonstrate in vitro that C-C mismatch on the 601 positioning sequence can enhance the mechanical stability of the nucleosome, previous studies have showed that the artificial 601 sequence do not preferentially or strongly position the nucleosomes in vivo as expected [27,28]. It is certainly desirable to perform mismatch-dependent nucleosome mechanics studies using native sequences but the fluorescence-force spectroscopy data of the type we acquired in this study would be difficult to interpret unless the nucleosomes are formed at a well-defined position. We recently reported a native sequence from the yeast gene SWH1 that forms a nucleosome in vitro centered at the dyad position in vivo[29].

While the enhancement of the mechanical stability of the nucleosome can potentially lead to the nucleosome's decreased accessibility for the mismatch repair mechanism, which can account for the accumulation of single nucleotide polymorphisms near the nucleosome dyad, other functional implications of the enhanced mechanical stability cannot be precluded. For example, enhanced mechanical stability might shield DNA from transcription, which prevents the expression of genes that contain misincorporated nucleotides. Another opportunity for future studies is the fate of oligonucleosomes under tension when one of the nucleosomes contains a mismatch.

Materials and Methods

Preparation of labeled DNA constructs

Each strand of DNA in constructs for cyclization measurements was prepared by ligation of two shorter DNA fragments containing labeled Cy3, Cy5 and biotin as indicated in Supplementary Figure S1. Typically, the fragments were mixed at the ratio of 1:1.2:1.5 for the first, the helper, and the second fragments for ligation with T4 DNA ligase (NEB) following the manufacture manual. The ligation mixture was then loaded on a denaturing PAGE gel to run electrophoresis for purification. We cut and chop the top band which had the correct length and let the DNA diffuse to a buffer containing 10 mM Tris pH 8 and 50 mM NaCl. After purification, the two complementary strands were annealed by mixng at 1:1 molar ratio and heating to 90°C followed by slow cooling over 3-4 hours. The final DNA construct contained the 601 sequence and was flanked by a 14 bp spacer to biotin for surface tethering and 20 bp spacer connect to a 12 nts overhang for annealing to lambda DNA.

Nucleosome preparation

Both *Xenopus laevis* and yeast histones were expressed in *E. coli*. Yeast histones were prepared in C. Wu's lab at the National Institutes of Health as described [30]. After purifying individual histone proteins, the histone octamers were prepared by denaturation-refolding and purification, according to standard procedures [31]. Xenopus histone octamers were purchased from The Histone Source, Colorado State University. To prepare nucleosomes, 601 DNA templates were reconstituted with the recombinant histone octamer by step-wise salt- dialysis [31]. The reconstituted nucleosome product was confirmed by an electrophoresis mobility shift assay for all experiments. Reconstituted nucleosomes were stored at 4°C in the dark, typically at concentrations of 100– 200 nM, and used within 4 weeks.

Single molecule DNA cyclization Measurement

DNA fragments for cyclization measurement were immobilized on a PEG-coated microscope slide via biotin-neutravidin linkage. The fragments had complementary 10 nt 5' overhang at either end, which permit looping via annealing. Cy3 and Cy5 were also present at the two 5' ends, resulting in high FRET in the looped state. Measuring FRET allowed us to quantify the fraction of looped molecules as a function of time since introduction of a high salt buffered solution (10 mM Tris-HCl pH 8.0, 1 M NaCl, 0.5% w/v D-Glucose (Sigma), 165 U/ml glucose oxidase (Sigma), 2170 U/ml catalase (Roche) and 3 mM Trolox (Sigma). The rate of loop formation was used as an operational measurement of DNA flexibility.

Force-Fluorescence Spectroscopy Measurement

We followed the protocol for force-fluorescence spectroscopy measurement published previously [14,15]. To construct the DNA tether for a Force-Fluorescence measurement, λ DNA was annealed to the reconstituted nucleosomes at one end, and to an oligonucleotide containing digoxigenin. The concentration of each element in the annealing reaction is 8 nM. During the experiment, the sample was diluted to 10 pM in nucleosome dilution buffer (10 mM Tris-HCl pH 8.0, 50 mM NaCl, 1 mM $MgCl_{2+}$ or 1 mM spermine) for immobilization on the PEG coated microscope slide. To attach the micro beads for optical trapping to the DNA construct. we diluted 1 µm anti-digoxigenin-coated polystyrene beads (Polysciences) in nucleosome dilution buffer and added it to the imaging chamber for 30 minutes. The fluorescence-force data acquisition procedures include three following steps using a custom built setup according to [16]. First, after trapping a bead, we determined the origin of the tether by stretching it in two opposite directions along x and y axis. Second, to spatially avoid beaching of the fluorophores, we displaced the trapped bead from its origin where the labeled nucleosome is located by 14 µm. To locate the exact position of the label nucleosomes for confocal acquisition of the fluorescence signal, we scan the

confocal laser around the tether's origin. Third, to apply the force on the tether, the nucleosome was stretched at a constant velocity of 455 nm/sec[1]. Fluorescence emission was recorded for 20 ms at each step during the stage movement by scanning the confocal excitation concurrently with the stage movement. Force-fluorescence data was obtained in imaging buffer (50 mM Tris-HCl pH 8, 50 mM NaCl, 1 mM $MgCl_2$ or Spermine, 0.5 mg/ml BSA (NEB), 0.5 mg/ml tRNA (Ambion), 0.1% v/v Tween-20 (Sigma), 0.5% w/v D-Glucose (Sigma), 165 U/ml glucose oxidase (Sigma), 2170 U/ml catalase (Roche) and 3 mM Trolox (Sigma)). tRNA was excluded in experiments with yeast-expressed nucleosomes.

All single molecule measurements were performed at the room temperature.

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Reviewer #1 (Public Review):

Summary:

In this manuscript, Ngo et al. report a peculiar effect where a single base mismatch (CC) can enhance the mechanical stability of a nucleosome. In previous studies, the same group used a similar state-of-the-art fluorescence-force assay to study the unwrapping dynamics of 601-DNA from the nucleosome and observed that force-induced unwrapping happens more slowly for DNA that is more bendable because of changes in sequence or chemical modification. This manuscript appears to be a sequel to this line of projects, where the effect of CC is tested. The authors confirmed that CC is the most flexible mismatch using the FRET-based cyclization assay and found that unwrapping becomes slower when CC is introduced at three different positions in the 601 sequence. The CC mismatch only affects the local unwrapping dynamics of the outer turn of nucleosomal DNA.

Strengths:

These results are in good agreement with the previously established correlation between DNA bendability and nucleosome mechanical stability by the same group. This well-executed, technically sound, and well-written experimental study contains novel nucleosome unwrapping data specific to the CC mismatch and 601 sequence, the cyclizability of DNA containing all base pair mismatches, and the unwrapping of 601-DNA from xenophus and yeast histones. Overall, this work will be received with great interest by the biophysics community and is definitely worth attention.

Weaknesses:

The scope and impact of this study are somewhat limited due to the lack of sequence variation. Whether the conclusion from this study can be generalized to other sequences and other bendability-enhancing mismatches needs further investigation.

Major questions:

(1) As pointed out by the authors, the FRET signal is not sensitive to nucleosome position; therefore, the increasing unwrapping force in the presence of CC can be interpreted as the repositioning of the nucleosome upon perturbation. It is then also possible that CC-containing DNA is not positioned exactly the same as normal DNA from the start upon nucleosome assembly, leading to different unwrapping trajectories. What is the experimental evidence that supports identical positioning of the nucleosomes before the first stretch?

(2) The authors chose a constant stretching rate in this study. Can the authors provide a more detailed explanation or rationale for why this rate was chosen? At this rate, the authors found hysteresis, which indicates that stretching is faster than quasi-static. But it must have been slow and weak enough to allow for reversible unwrapping and wrapping of a CC-containing DNA stretch longer than one helical turn. Otherwise, such a strong effect of CC at a single location would not be seen. I am also curious about the biological relevance of the magnitude of the force. Can such force arise during nucleosome assembly in vivo?

(3) In this study, the CC mismatch is the only change made to the 601 sequence. For readers to truly appreciate its unique effect on unwrapping dynamics as a base pair defect, it would be nice to include the baseline effects of other minor changes to the sequence. For example, how robust is the unwrapping force or dynamics against a single-bp change (e.g., AT to GC) at the three chosen positions?

(4) The last section introduces yeast histones. Based on the theme of the paper, I was expecting to see how the effect of CC is or is not preserved with a different histone source. Instead, the experiment only focuses on differences in the unwrapping dynamics. Although the data presented are important, it is not clear how they fit or support the narrative of the paper without the effect of CC.

(5) It is stated that tRNA was excluded in experiments with yeast-expressed nucleosomes. What is the reason for excluding it for yeast nucleosomes? Did the authors rule out the possibility that tRNA causes the measured difference between the two nucleosome types?

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Reviewer #2 (Public Review):

Summary:

Mismatches occur as a result of DNA polymerase errors, chemical modification of nucleotides, during homologous recombination between near-identical partners, as well as during gene editing on chromosomal DNA. Under some circumstances, such mismatches may be incorporated into nucleosomes but their impact on nucleosome structure and stability is not known. The authors use the well-defined 601 nucleosome positioning sequence to assemble nucleosomes with histones on perfectly matched dsDNA as well as on ds DNA with defined mismatches at three nucleosomal positions. They use the R18, R39, and R56 positions situated in the middle of the outer turn, at the junction between the outer turn and inner turn, and in the middle of the inner turn, respectively. Most experiments are carried out with CC mismatches and Xenopus histones. Unwrapping of the outer DNA turn is monitored by single-molecule FRET in which the Cy3 donor is incorporated on the 68th nucleotide from the 5'-end of the top strand and the Cy5 acceptor is attached to the 7th nucleotide from the 5' end of the bottom strand. Force is applied to the nucleosomal DNA as FRET is monitored to assess nucleosome unwrapping. The results show that a CC mismatch enhances nucleosome mechanical stability. Interestingly, yeast and Xenopus histones show different behaviors in this assay. The authors use FRET to measure the cyclization of the dsDNA substrates to test the hypothesis that mismatches enhance the flexibility of the 601 dsDNA fragment and find that CC, CA, CT, TT, and AA mismatches decrease looping time, whereas GA, GG, and GT mismatches had little to no effect. These effects correlate with the results from DNA buckling assays reported by Euler's group (NAR 41, 2013) using the same mismatches as an orthogonal way to measure DNA kinking. The authors discuss that substitution rates are higher towards the middle of the nucleosome, suggesting that mismatches/DNA damage at this position are less accessible for repair, consistent with the nucleosome stability results.

Strengths:

The single-molecule data show clear and consistent effects of mismatches on nucleosome stability and DNA persistence length.

Weaknesses:

It is unclear in the looping assay how the cyclization rate relates to the reporting looping time. The biological significance and implications such as the effect on mismatch repair or nucleosome remodelers remain untested. It is unclear whether the mutational pattern reflects the behavior of the different mismatches. Such a correlation could strengthen the argument that the observed effects are relevant for mutagenesis.

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Reviewer #3 (Public Review):

Summary:

The mechanical properties of DNA wrapped in nucleosomes affect the stability of nucleosomes and may play a role in the regulation of DNA accessibility in eukaryotes. In this manuscript, Ngo and coworkers study how the stability of a nucleosome is affected by the introduction of a CC mismatched base pair, which has been reported to increase the flexibility of DNA. Previously, the group has used a sophisticated combination of single-molecule FRET and force spectroscopy with an optical trap to show that the more flexible half of a 601 DNA segment provides for more stable wrapping as compared to the other half. Here, it is confirmed with a single-molecule cyclization essay that the introduction of a CC mismatch increases the flexibility of a DNA fragment. Consistent with the previous interpretation, it also increased the unwrapping force for the half of the 601 segment in which the CC mismatch was introduced, as measured with single-molecule FRET and force spectroscopy. Enhanced stability was found up to 56 bp into the nucleosome. The intricate role of mechanical stability of nucleosomes was further investigated by comparing force-induced unwrapping profiles of yeast and Xenopus histones. Intriguingly, asymmetric unwrapping was more pronounced for yeast histones.

Strengths:

(1) High-quality single-molecule data.

(2) Novel mechanism, potentially explaining the increased prominence of mutations near the dyads of nucleosomes.

(3) A clear mechanistic explanation of how mismatches affect nucleosome stability.

Weaknesses:

(1) Disconnect between mismatches in nucleosomes and measurements comparing Xenopus and yeast nucleosome stability.

(2) Convoluted data in cyclization experiments concerning the phasing of mismatches and biotin site.

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