Dependence of Nucleosome Mechanical Stability on DNA Mismatches and Histone Variants

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Abstract

The organization of nucleosomes into chromatin and their accessibility are shaped by local DNA mechanics and modulated by histone variants. Conversely, nucleosome positions shape genetic variations, which may originate from mismatches during replication and chemical modification of DNA. To investigate how DNA mismatches and histone variants 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 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 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. Yeast nucleosomes are mechanically less stable and more symmetrical in the outer turn unwrapping compared to Xenopus nucleosomes. H2A.Z histone variants display no measurable difference in mechanical unwrapping patterns compared to canonical yeast nucleosomes. 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.

Introduction

DNA base-base mismatches are generated by nucleotide misincorporation during DNA synthesis or by chemical modification such as hydrolytic deamination of cytosine [1]. 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 the 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 bps 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.

Histone variants play a major role in chromatin organization and functions [14,15]. In higher eukaryotes histone H3 has two major variants, H3.1 and H3.2, which differ in their timing of DNA deposition whereas in yeast, there is only one histone H3 which is more similar to H3.2 than H3.1. *In vitro* biochemical assay with histones from different species expressed in bacteria, yeast, and xenopus, sometimes resulted in observations difficult to reconcile with *in vivo* findings [16-18]. Among histone variants, H2A.Z is an evolutionarily conserved histone that plays a critical role in many diverse functions [19,20]. H2A.Z is commonly found at active promoters and enhancers [21,22]. *In vivo* assays suggested different effects of H2A.Z on nucleosome stability and mobility, depending on the context [19,23,24]. *In vitro* assays

demonstrated that H2A.Z nucleosomes are more salt sensitive and less stable than canonical nucleosomes [25,26]. Inside the cell, nucleosomes are likely to be under mechanical forces but how mechanical forces may influence nucleosomal DNA accessibility for nucleosomes containing histone variants is not known.

In an earlier work, we demonstrated a correlation between DNA flexibility and nucleosome stability under tension using the 601 nucleosome positioning sequence [27]. We showed that the 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 [28]. Here, we examined the effect of 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 with canonical H2A and H2A.Z variants.

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 [29-31]. 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 mismatch 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). 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 [27], 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 [27,28] and a more recent study [32] 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.

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 [27], 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 for mismatch-containing nucleosomes. 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 [33]. 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.

DNA mismatch enhances nucleosome mechanical stability

We compared the FRET vs 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. 3 C-E). Fig. 4 shows the averaging of FRET vs. Force for different constructs. 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 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). The effect was the highest for the mismatch placing at the junction of the inner turn and outer turn (R39).

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 closed 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 [27]. 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 mismatches enhance 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 bendability. Therefore, we used a single

molecule DNA cyclization assay [34] 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 closed 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 [35,36]. 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 at three different positions reduced the looping time from 57min 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 [37]. 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 [37]. 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 mismatch makes the construct loop faster, and that this is not an artifact specific to the biotin tether location.

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 of 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. 6), 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. 6A-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. 6C). 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 when tension is applied than nucleosomes made with Xenopus histones.

Yeast histone variant H2A.Z does not alter nucleosome mechanical stability

Previous *in vivo* assays suggested that H2A.Z nucleosomes may increase or decrease nucleosome mobility and stability depending on the context [23,26]. *In vitro* biochemical assays using native or bacterially expressed histones demonstrated that H2A.Z nucleosomes are less stable than H2A nucleosomes [16,17,25]. We sought to examine how H2A.Z affect nucleosome mechanically stability using fluorescence-force spectroscopy. The same DNA constructs with the outer turn probes, ED1 and ED2, and inner turn probe INT were reconstituted with yeast histone octamer containing canonical or H2A.Z histones. Both canonical and H2A.Z nucleosomes displayed similar zero-force FRET values for all three probes ED1, ED2 and INT, suggesting there is no measurable difference in the positioning of the nucleosomes with the H2A.Z variant compared to canonical histones (Fig. 7). Upon applying pulling force, the averaged unwrapping trajectories probed by the outer turn probe ED1 and ED2 are similar for canonical and H2A.Z nucleosomes (Fig. 7A-B). Internal INT probe also reported similar stretching patterns for the H2A.Z variant and canonical nucleosomes (Fig. 7C). In summary, we observed no significant difference in nucleosome mechanical stability between yeast nucleosomes containing H2A.Z or H2A histones for the 601 positioning sequence.

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 to DNA conformational dynamics using other methods such as NMR [4] and the DNA Euler buckling assay [3]. 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 nucleosome reconstituted from a mismatch DNA construct. 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 [27,28].

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 [27]. The 601 nucleosome is more stable on the TA-rich side [27,32,38], 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 [27], 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 repeatswhere which side unwraps at low forces became stochastic [27], 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 apparent flexibility 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 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 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 [27,28], we can predict that other DNA lesions and alternative DNA structures such as DNA single strand damages, bulky adducts and R-loops [39] 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. These observations may indicate a mechanical role of species-to-species differences in histone sequence and post-translation modifications, which need to be examined in future studies. In contrast, yeast H2A.Z nucleosome did not display measurable difference in FRET and stretching pattern compared to canonical yeast nucleosomes. This observation appears to be consistent with the biochemical behavior of bacterially expressed H2A.Z nucleosomal histones [40], but is inconsistent with the reduced stability of mammalian H2A.Z nucleosomes reconstituted on their native DNA sequences when subjected to a pulling force in a single-molecule assay [25]. Our mechanical measurement on reconstituted nucleosomes also do not explain the source of the salt sensitivity of natural H2A.Z histones in nuclei containing a variety of post-translational modifications [16,17,25,41]. Further studies are needed to clarify whether the experimental inconsistencies are related to the differences between natural and bacterially expressed histones, to

sequence differences between yeast and mammalian H2A.Z, to the use of native DNA sequences or positioning sequences, or to the experimental nuances of distinct single-molecule mechanical assays.

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 to 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^{o}C followed by slow cooling over 3-4 hours. The final DNA construct contained the 601 sequence and 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 [42]. After purifying individual histone proteins, the histone octamers were prepared by denaturation-refolding and purification, according to standard procedures [43]. 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 [43]. The reconstituted nucleosome product was confirmed by an electrophoresis mobility shift assay for all experiments. Reconstituted nucleosomes were stored at 4^{o}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 [27,28]. 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 [29]. 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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Figures

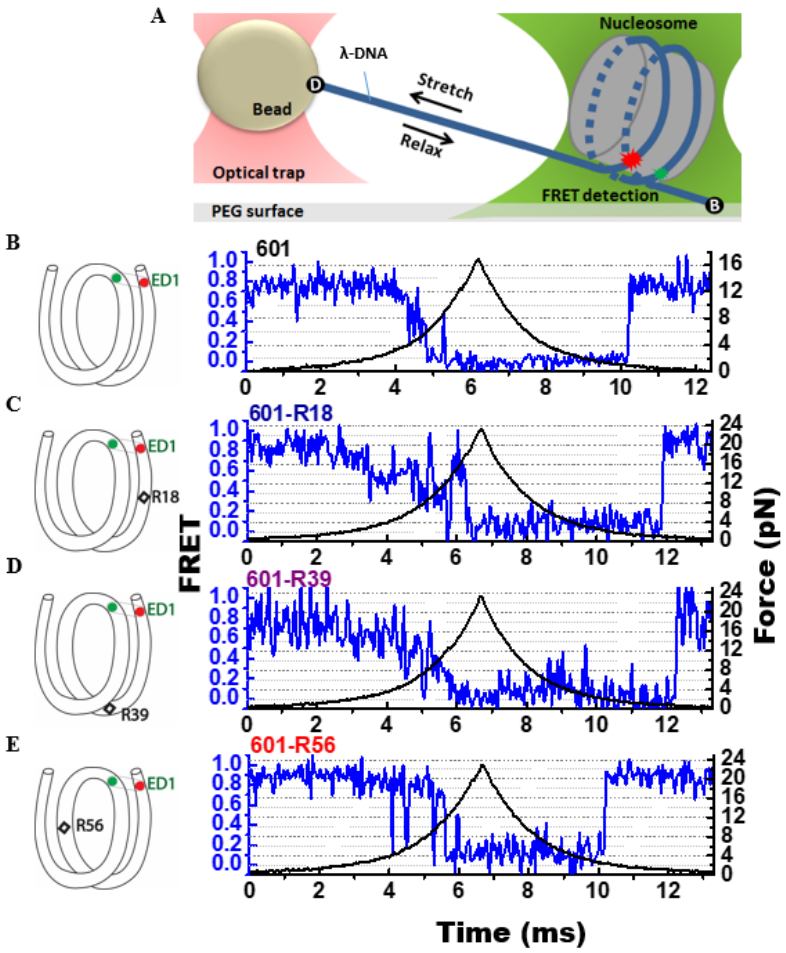


Figure 1: Nucleosome unwrapping measurement. (**A**) Experimental scheme: (**B, C, D, E**): Representative stretching traces of the outer turn (ED1) for nucleosomes reconstituted from the 601 sequence (**B**) and from the 601 sequence with containing a mismatch at different positions: on the outer turn (**C**), at the junction of the outer turn and inner turn (**D**) and at the inner turn (**E**).

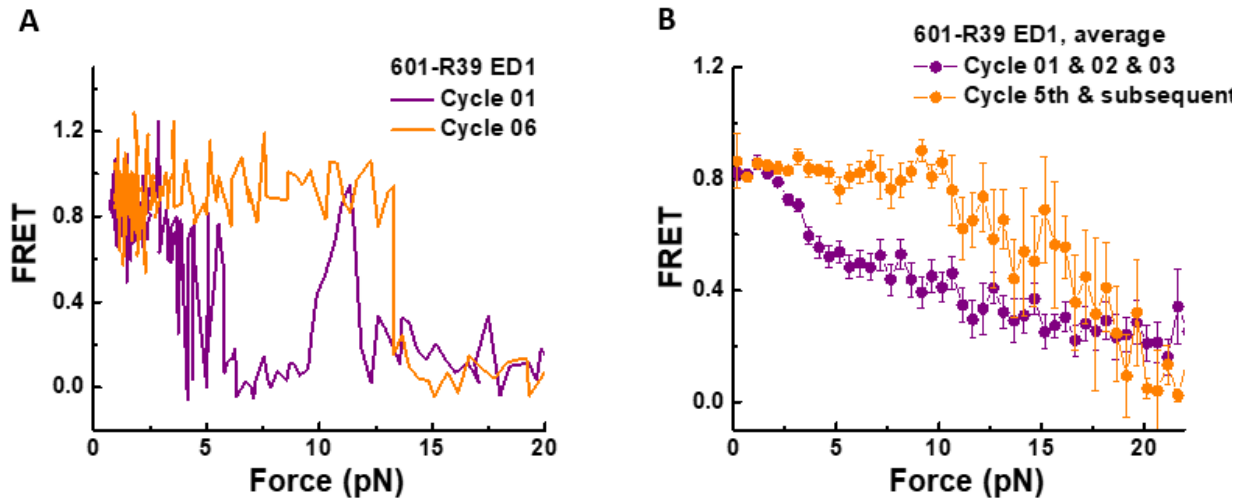


Figure 2: Unwrapping force of mismatch-containing nucleosomes is higher for subsequent stretching cycles. (A) Representative single-molecule stretching traces at two stretching cycles from the sample molecule, probe by the ED1 FRET pair. (**B**) Averaging FRET vs. Force for many molecules at the first 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.

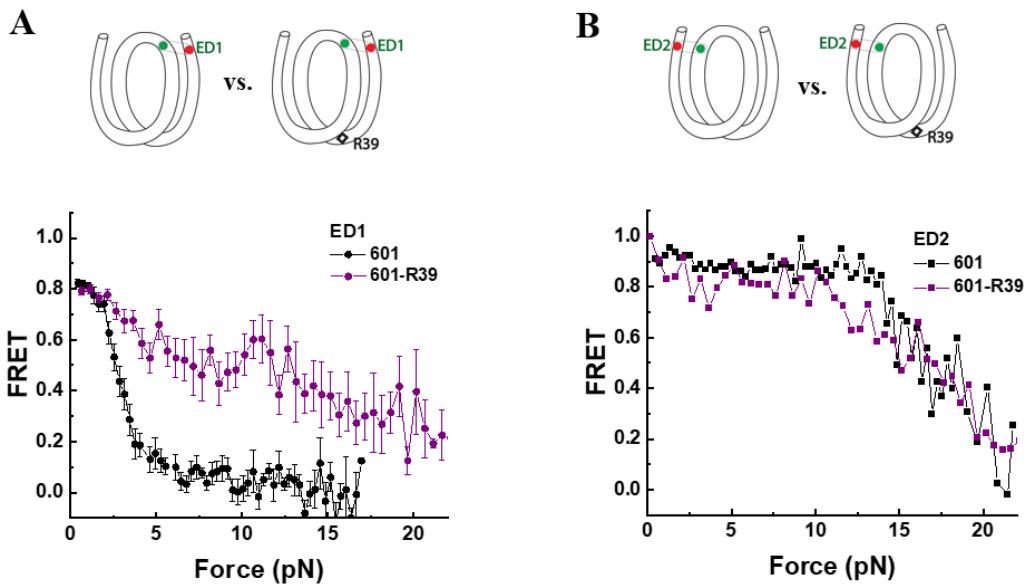


Figure 3: Enhancement of nucleosome mechanical stability by DNA mismatch. Average of FRET vs. Force for ED1 probe (**A**) and ED2 probe (**B**) for the 601 nucleosome (black) and for the first stretching cycle of the mismatch containing nucleosome 601-R39 (purple). Histone proteins were expressed in 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.

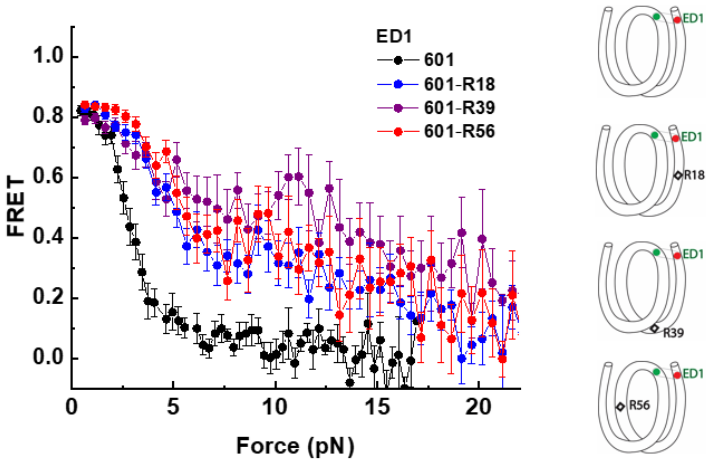


Figure 4: Mismatch position-dependence of nucleosome unwrapping. Average of FRET vs. Force for ED1 probe for the 601 nucleosome (black) and the mismatch-containing nucleosome 601-R39 (purple), 601-R18 (blue) and 601-R56 (red). Histone proteins were expressed in xenopus. The error bars represent S.D. of n = 25, 11, 7 and 10 for the 601, 601-R18, 601-R39 and 601-R56 nucleosomes, respectively.

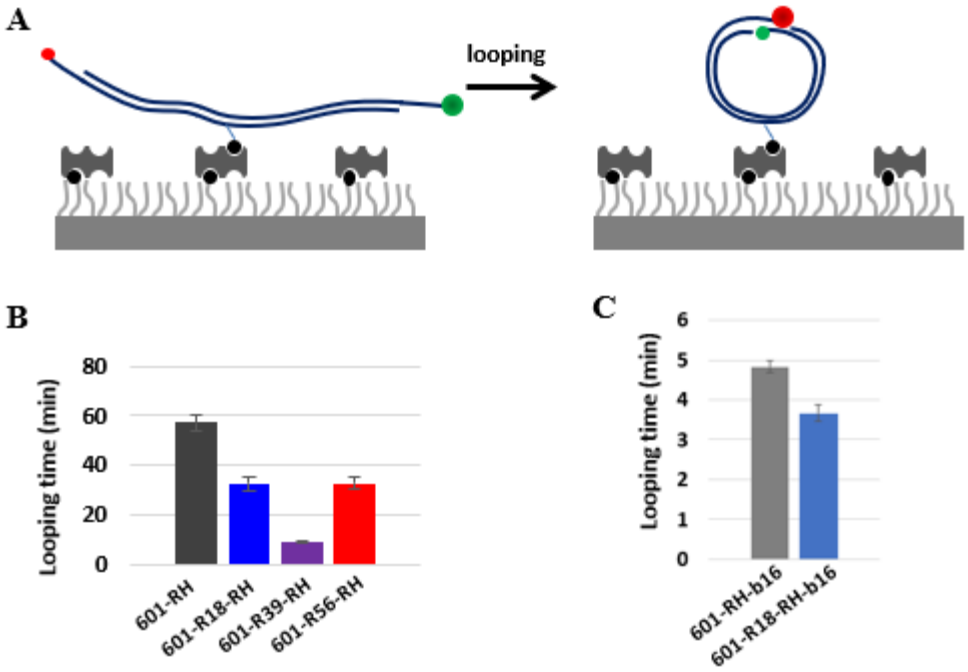


Figure 5: DNA mismatches enhance DNA flexibility. (**A**): Single molecule cyclization assay: The DNA construct with 10-nucleotide complementary sticky ends is immobilized on a PEG passivated imaging chamber. DNA looping is induced using the imaging buffer containing 1M NaCl followed by time course TIRF 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.

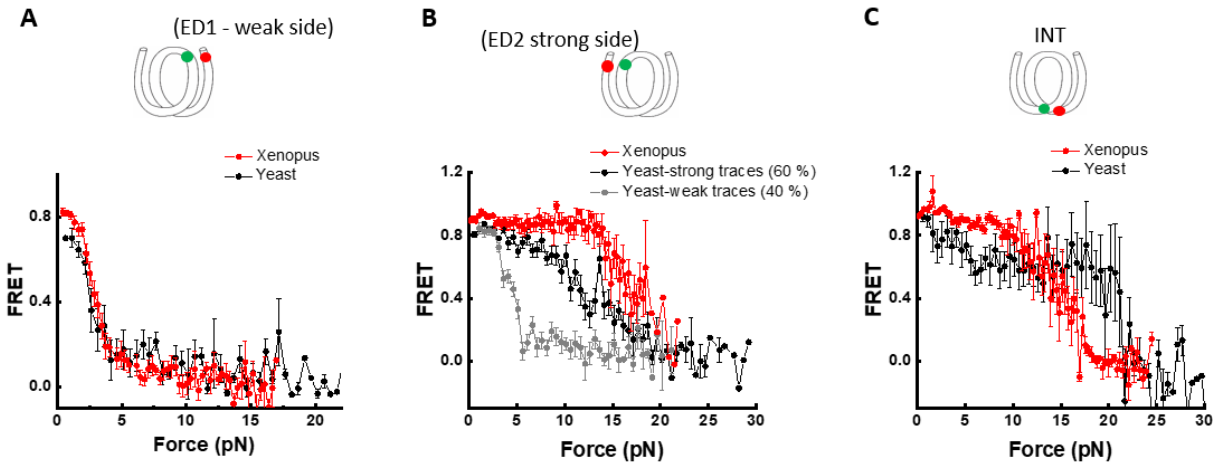


Figure 6: Unwrapping of yeast vs. xenopus reconstituted nucleosomes: Average of FRET vs. Force for nucleosomes reconstituted from xenopus (red) vs yeast (black and gray) histone proteins with DNA labeled by outer turn probes ED1 (**A**), ED2 (**B**) and inner turn probe INT (**C**). The error bars represent 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.

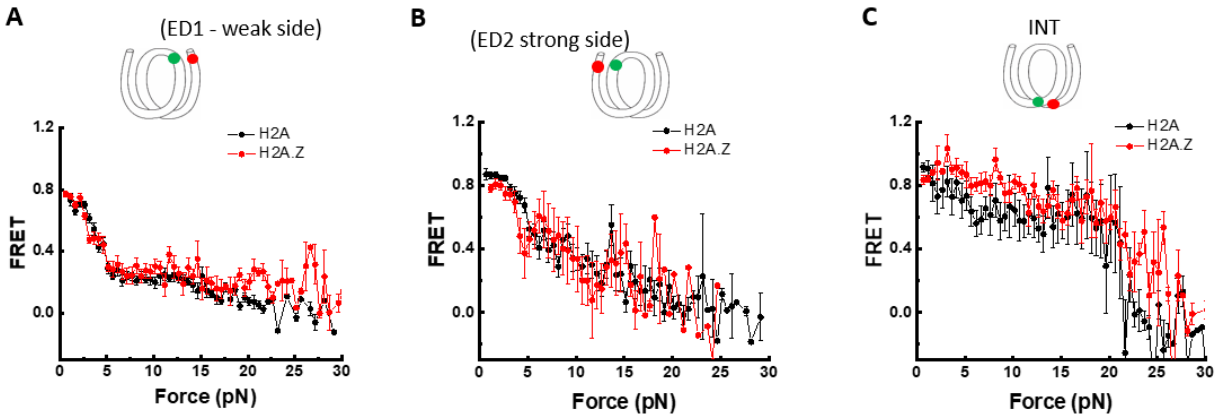
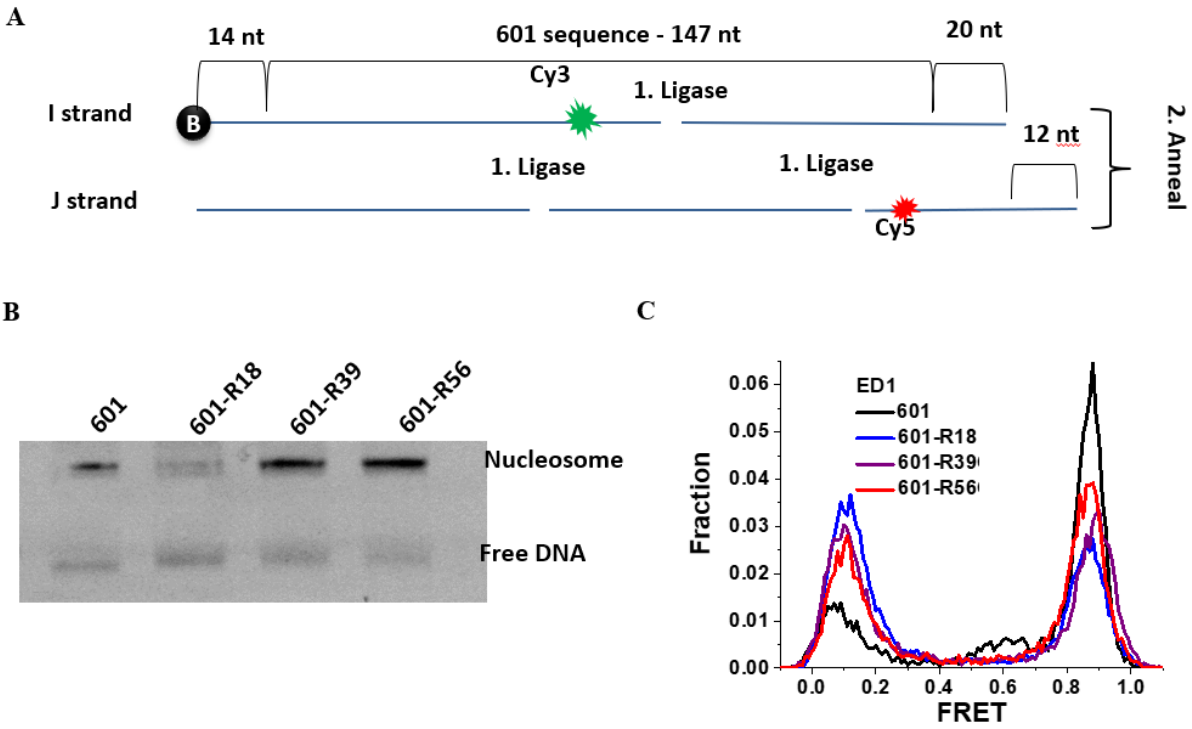


Figure 7: Unwrapping of H2A. Z vs. canonical H2A nucleosomes: Average of FRET vs. Force for nucleosomes reconstituted from yeast histone octamer containing H2A.Z variant (red) and canonical

H2A (black) 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 = 5 (H2A) and 5 (H2A.Z) nucleosomes with the ED1 probe (**A**), n = 10 (H2A) and 4 (H2A.Z) with the ED2 probe (**B**), and n = 6 (H2A) and 7 (H2A.Z) nucleosomes with the INT probe (**C**), respectively.

Supplementary Figures



Supplementary Figure S1: Nucleosome preparation

(A) Scheme of the DNA template prepared by ligation of short, labeled oligos

(B) Migration of the 601 nucleosome mismatch containing nucleosomes on 5% native PAGE

FRET histogram of the 601 nucleosome mismatch containing nucleosomes with ED1 labeling scheme.