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Communication

Histone H2A Ubiquitination Reinforces Mechanical Stability and Asymmetry at the Single-Nucleosome Level

Xue Xiao,[▽] Cuifang Liu,[▽] Yingxin Pei,[▽] Yi-Zhou Wang, Jingwei Kong, Ke Lu, Lu Ma, Shuo-Xing Dou, Peng-Ye Wang, Guohong Li, Ping Chen,* and Wei Li*

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ABSTRACT: Monoubiquitination at lysine 119 of histone H2A (ubH2A) is a prevalent post-translational modification that is associated with gene repression in the context of chromatin. However, the direct function of ubH2A on nucleosome is poorly understood. Here we identified the effect of ubH2A on nucleosome using single-molecule magnetic tweezers. We revealed that ubH2A stabilizes the nucleosome by blocking the peeling of DNA from the histone octamer. Each ubH2A reinforces one-half of the outer wrap and introduces a robust asymmetry for nucleosome unfolding. Furthermore, a real-time deubiquitination process confirmed that ubH2A-nucleosome is sequentially deubiquitinated and restored to the unmodified nucleosome state. These results provide a novel mechanism to understand the repression of the passage of RNA or DNA polymerases through the ubH2A-nucleosome barrier during gene transcription or replication.

Eukaryotic DNA in cells is packaged into chromatin to be stored in the nucleus. Nucleosome, the fundamental unit of chromatin, is composed of 147 base pairs (bp) of DNA wrapped around the octamer of four core histones (H2A, H2B, H3, and H4) about 1.65 left-hand turns.[1] All DNA-related biological processes, including DNA transcription and replication, occur in the context of chromatin. The nucleosome barrier must be temporarily removed first to facilitate access to the DNA template and then rapidly restored afterward. Post-translational modifications of core histones, such as acetylation, methylation, and ubiquitination are involved in these regulations of nucleosome dynamics.[2,3]

Ubiquitination is a major histone modification in mammalian cells in which the 76 amino acid protein ubiquitin is covalently attached to the ϵ-amino group of a lysine residue. H2A is the first histone identified as being ubiquitinated, and the ubiquitination site predominately occurs on the highly conserved residue lysine 119.[4,5] About 5−15% of total H2A has been reported to be monoubiquitinated at lysine 119 (ubH2A) in higher eukaryotic organisms. ubH2A has been reported to be tightly associated with transcription regulation and DNA damage repair.[6,7] Most of our knowledge about the function of ubH2A derives from studies of polycomb repressive complex (PRC1), the major H2A ubiquitinase.[8] ubH2A has been found to prevent FACT recruitment and block release of RNA polymerase II.[9] Muir et al. demonstrated a mild nucleosome stabilizing effect of ubH2A based on Nap1-mediated nucleosome formation by fluorescence resonance energy transfer (FRET).[10] This evidence reveals a strong connection between ubH2A and gene expression. To date, however, the direct functions of ubH2A on nucleosomes are still poorly understood.

Nucleosomes with human ubH2A (ubH2A-nucleosome) or unmodified H2A (H2A-nucleosome) were reconstituted in vitro onto a 409 bp DNA fragment containing one Widom 601 nucleosome positioning sequence (Figures 1a and S1). All of the nucleosomes were well-positioned on the middle 601 sequence, as characterized by gel electrophoresis and atomic force microscopy (AFM) imaging (Figure S1). For H2A-nucleosome, the well-defined two-step unfolding dynamics was revealed by the use of magnetic tweezers (Figure 1c, top panel), with the outer nucleosome DNA wrap disassembled at ∼5 pN and the inner wrap disassembled at ∼25 pN, which is consistent with previous studies by us[11,12] and others.[13,14] Interestingly, ubH2A-nucleosome was observed to disassemble at much higher forces of ∼20 pN for the outer wrap and ∼32 pN for the inner wrap (Figure 1c, bottom panel). The rupture forces for the outer and inner wraps were statistically characterized for hundreds of samples (Figure 1d). ubH2A-nucleosomes obtain much higher mechanical stability than H2A-nucleosomes. The stabilization effect of ubH2A on the salt-dependent stability of nucleosomes was also investigated by FRET analyses, and ubH2A was found to increase the unfolding midpoint to 0.67 ± 0.01 M NaCl, compared with 0.43 ± 0.01 M NaCl for H2A-nucleosomes (Figure S2).

To quantify the stabilization effect of ubH2A, we measured the free energy cost for unfolding the outer DNA wrap. For H2A-nucleosome, the two-state transitions of the outer wrap were observed with a calculated free energy cost of 32 kJ/mol (Figure 1e; calculation details are provided in the Supporting Information), consistent with previous studies.[11,13] Importantly, for ubH2A-nucleosome, the unfolding dynamics of the outer DNA wrap is divided into two 10 nm steps with different

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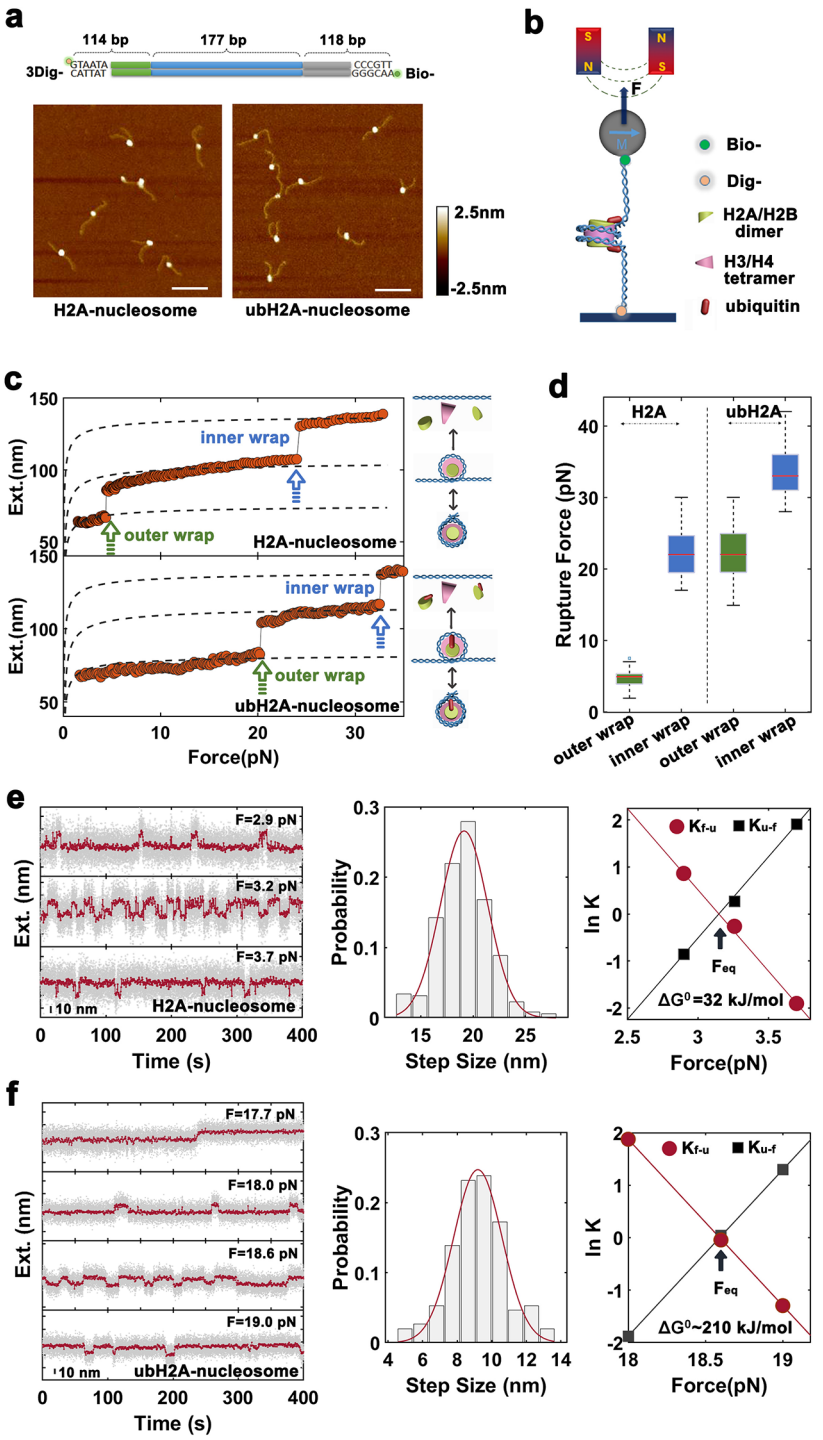

Figure 1. ubH2A reinforces the mechanical stability of nucleosome. (a) AFM images of (left) H2A- and (right) ubH2A-nucleosome reconstituted on the DNA template shown at the top. Scale bars: 100 nm. (b) Schematic setup of the magnetic tweezers. (c) Typical force−extension curves of (top) H2A-nucleosome and (bottom) ubH2A-nucleosome. The dashed lines indicate three states of two-step unfolding force−extension curves fitted with 262, 320, and 409 bp free DNA, respectively, using the wormlike chain (WLC) model. (d) Statistical rupture forces for the outer and inner DNA wraps for (left) H2A- and (right) ubH2A-nucleosome. (e, f) Folding/unfolding trajectories of the outer DNA wrap at various tensions (left), distributions of extension jump (middle), and relationship between the kinetic rate constant and tension (right) for (e) H2A-nucleosome and (f) ubH2A-nucleosome.

modes (Figures 1f and S3). The first one-step transition at 17.7 pN corresponds to the disruption of one half of the outer DNA wrap, with an evaluated free energy of 105 kJ/mol. The second half of the outer DNA wrap presents a two-state transition with a calculated free energy of 106 kJ/mol. For ubH2A-nucleosome, the total free energy cost of outer wrap unfolding at zero tension is ~210 kJ/mol. ubH2A gives rise to different folding kinetics as well as dramatically enhances the mechanical stability of nucleosome.

There are two copies of ubH2A in one nucleosome. How do they correlate with each other to affect the stability of nucleosome? The hybrid nucleosome with one ubH2A and

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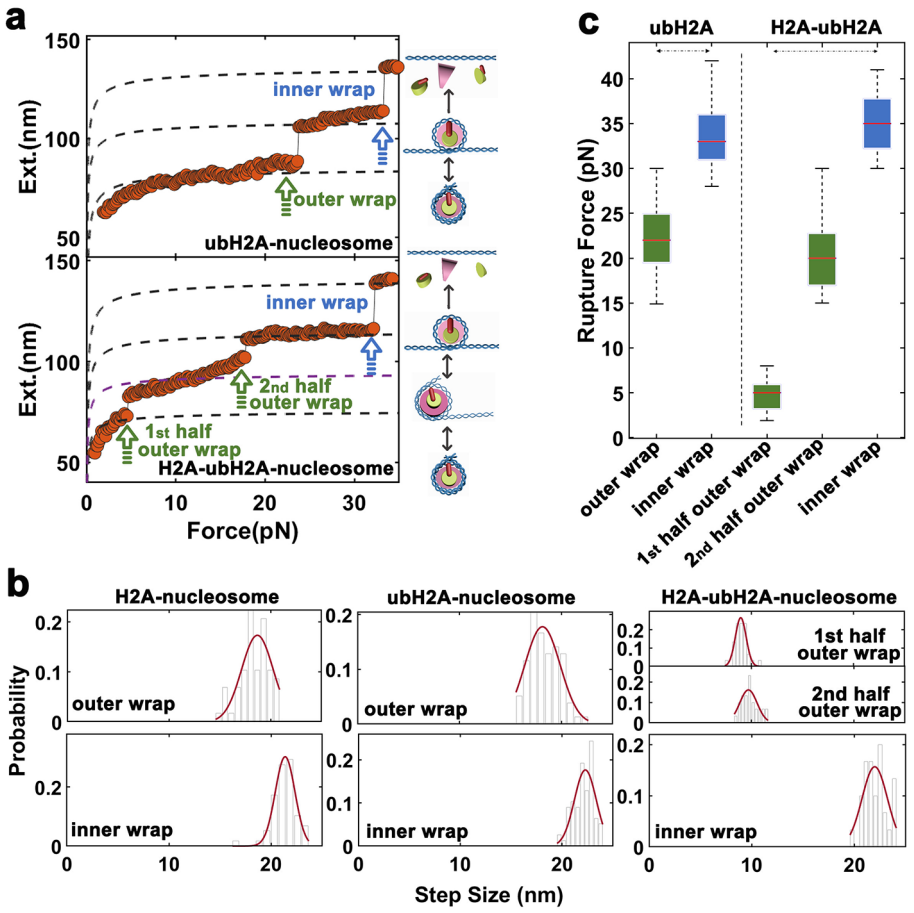

Figure 2. One ubH2A stabilizes one half of the outer DNA wrap and introduces a robust unfolding asymmetry for the H2A-ubH2A-nucleosome. (a) Typical force−extension curves of (top) ubH2A-nucleosome and (bottom) H2A-ubH2A-nucleosome. The dashed lines indicate four states of H2A-ubH2A-nucleosome in the force−extension curve fitted with 262, 291, 320, and 409 bp free DNA using the WLC model. (b) Step-size distributions of the outer and inner wraps for H2A-, ubH2A-, and H2A-ubH2A-nucleosome. (c) Statistical measurements of rupture forces for the outer and inner DNA wraps for (left) ubH2A-nucleosome and (right) H2A-ubH2A-nucleosome.

one unmodified H2A (H2A-ubH2A-nucleosome) was reconstituted (Figure S4). Surprisingly, in contrast to the ubH2A-nucleosomes, which are disassembled totally within two steps under tension of more than 20 pN (Figure 2a, top panel), H2A-ubH2A-nucleosomes display distinct three-step unfolding dynamics (Figure 2a, bottom panel). The first two 10 nm steps at forces of around 5 and 20 pN correspond to the unraveling of the two halves of the outer DNA wrap, and the third 25 nm step at a force of around 30 pN corresponds to the unraveling of the inner wrap. The 20 nm one-step disruption of the outer DNA wrap in ubH2A-nucleosome is split into two 10 nm steps for H2A-ubH2A-nucleosome (Figure 2b). The rupture forces for the outer and inner DNA wraps were statistically characterized (Figure 2c). These results revealed that one ubH2A stabilizes one half of the outer DNA wrap and introduces an obvious asymmetry for nucleosome unfolding.

How does the ubH2A dramatically stabilize nucleosome? The ubiquitination site lysine 119 is located at the beginning of the C-terminal tails of H2A and at the end of H2A docking domain.[1] Two possibilities may account for the stabilizing effect of ubH2A: ubiquitin may directly bind to DNA or histones on nucleosome or ubH2A may stabilize the octamer by enhancing the interaction between the H2A/H2B dimer and H3/H4 tetramer. To clarify this, we investigated the interactions of ubiquitin with histones and DNA. We found that ubiquitin does not directly bind to the four core histones and binds to DNA very weakly (Figure 3a,b). The binding capacity of ubiquitin to DNA is several hundred times weaker than that for linker histone H1, which binds to nucleosome in the vicinity of the H2A ubiquitination site (Figure S5a). However, ubH2A displays a much greater stabilizing effect on the outer DNA wrap, with a disruption force of around 20 pN, compared with linker histone H1, which stabilizes the wrap with a disruption force of around 10 pN.[11] These results indicate that the stabilizing effect of ubH2A is not caused by the direct binding of ubiquitin to DNA or histones on nucleosome.

We further investigated whether the stabilizing effect of ubH2A is caused by enhancement of the stability of the octamer by ubH2A. Previous investigation showed that the cysteine cross-link between the two copies of H2A via H2A-N38C greatly stabilizes the octamer (Figure S5b) but does not affect the interaction between the octamer and nucleosomal DNA.[15] We reconstituted the nucleosome with the cross-linked H2A-N38C (crH2A-nucleosome) (Figure 3c) and found that the trajectories of crH2A-nucleosome were similar to that of normal H2A-nucleosome (Figure 3d), which indicated that the stabilized octamer does not affect the nucleosome unfolding. We further ubiquitinated crH2A-nucleosome specifically at lysine 119 by in vitro ubiquitination

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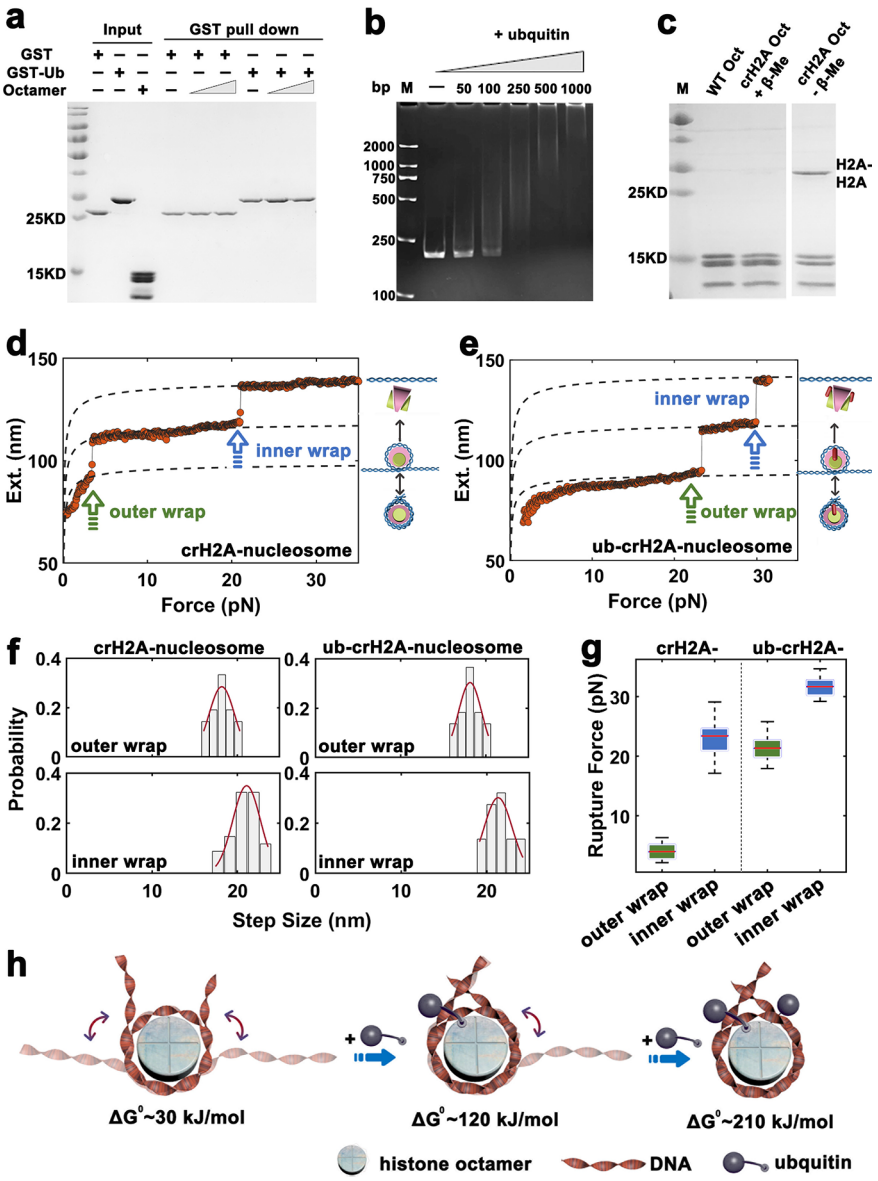

Figure 3. Ubiquitination of H2A stabilizes the nucleosome by blocking the peeling of DNA from the octamer. (a) No direct interaction between ubiquitin and histones is observed by GST-pull down assay. Input corresponds to 10% of related proteins. (b) Gel-shift assay showing the DNA binding properties of ubiquitin. The first lane is the marker, and the next lanes show DNA incubated with different concentrations of ubiquitin at the indicated protein/DNA molar ratios. (c) SDS-PAGE analysis of the purified histone octamers with wild-type H2A (WT Oct), non-cross-linked H2A-N38C (crH2A Oct+β-Me), and cross-linked H2A-N38C (crH2A Oct-β-Me). (d, e) Typical force−extension curves of (d) crH2A-nucleosome and (e) ub-crH2A nucleosome. (f) Step-size distributions of the outer and inner wraps for (left) crH2A- and (right) ub-crH2A-nucleosomes. (g) Statistical measurements of rupture forces for the outer and inner DNA wraps for (left) crH2A- and (right) ub-crH2A-nucleosomes. (h) Proposed model for the stabilizing effect of ubH2A on nucleosome. The ubiquitin modified on H2A acts as a bolt to block the peeling of DNA from the octamer.

assay (Figure S5c) and found that the disruption trajectories of ub-crH2A-nucleosome (Figure 3e), the step size distributions (Figure 3f), and the statistical measurements of rupture forces (Figure 3g) for the outer and inner DNA wraps are similar to those of ubH2A-nucleosome (Figures 1c, 2b, and 1d). These results revealed that the stabilizing effect of ubH2A is also not caused by enhanced stability of the octamer. ubH2A reinforces the mechanical stability of nucleosome by the specific location of ubiquitin on the nucleosome, where the ubiquitin may act as a bolt to block the peeling of DNA from the octamer (Figure 3h).

To confirm these effects of ubH2A on nucleosome, we traced the real-time deubiquitinating process of USP21 on ubH2A-nucleosomes. USP21 has been identified as a deubiquitinase for ubH2A.[16] Our results showed that ubH2A-nucleosomes were successfully deubiquitinated by USP21 with high efficiency (Figure 4a), and the disruption trajectories of the deubiquitinated ubH2A-nucleosomes were restored to those for H2A-nucleosome (Figure 4b). The real-time deubiquitinating process of USP21 on ubH2A was traced using magnetic tweezers. For ubH2A-nucleosomes, no structural disruptions were observed as the tension was continuously increased to 7 pN (Figure 4c, middle panel). After the injection of USP21, two sequential 10 nm disruptions occurred, corresponding to the unfolding of the two halves of the outer wrap. For H2A-ubH2A-nucleosome (Figure 4c,

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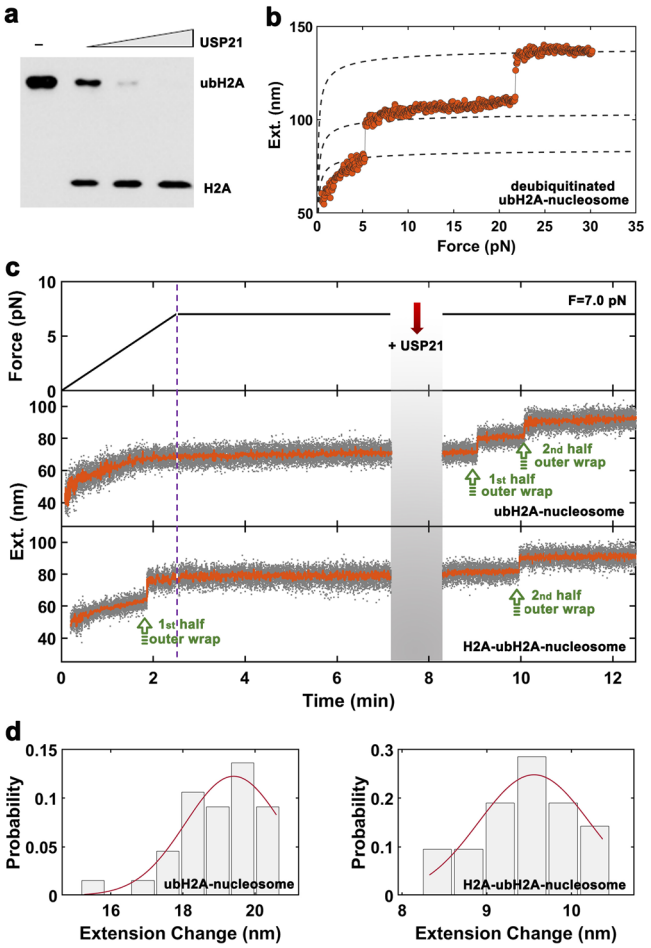

Figure 4. Deubiquitination of ubH2A-nucleosome and H2A-ubH2A-nucleosome by USP21 diminishes the stabilizing effect of ubH2A. (a) Western blot experiments showing that ubH2A-nucleosomes are successfully deubiquitinated by USP21 with high efficiency. (b) Force–extension curves of deubiquitinated ubH2A-nucleosome. (c) Real-time traces of the deubiquitination processes for ubH2A-nucleosome (middle) and H2A-ubH2A-nucleosome (bottom). The tension was first increased to 7.0 pN continuously at 0.05 pN/s and then held at 7.0 pN for 5 min before the injection of USP21 (top). (d) Distributions of the extension change after injection of USP21 into the flow cell for (left) ubH2A- and (right) H2A-ubH2A-nucleosome.

bottom panel), a 10 nm extension jump was observed as the tension increased to 7 pN, which corresponds to disruption of the weak interaction of unmodified H2A with DNA. After the injection of USP21, the disruption of the second half of the outer DNA wrap was identified, which corresponds to peeling of DNA from the ubH2A side of the nucleosome. The total extension changes for ubH2A- and H2A-ubH2A-nucleosomes after the injection of USP21 were statistically characterized (Figure 4d). After deubiquitination, both the ubH2A and H2A-ubH2A-nucleosome were restored to H2A-nucleosome (Figure S6). The real-time deubiquitination investigations provide clear evidence that one ubH2A reinforces the mechanical stability of one half of the outer wrap of nucleosome.

In this study, we investigated the direct effect of ubH2A on nucleosome. We found that ubH2A greatly stabilizes the outer DNA wrap of nucleosome, with one ubH2A stabilizing one half of the outer wrap. Deubiquitination of ubH2A by USP21 can relieve this stabilization and restore the stability and dynamics to the state of H2A-nucleosome. The ubiquitin does not directly bind to histones and very weakly binds to DNA. We conclude that ubH2A blocks the peeling of DNA from the octamer to stabilize the nucleosome, which may function to repress the passage of RNA or DNA polymerases through the nucleosome barrier during gene transcription or replication. Each ubH2A reinforces one half of outer wrap, which indicates that besides the DNA flexibility as pointed by Ngo et al.,[17] asymmetric ubiquitination of H2A in vivo may also introduce asymmetric folding dynamics of nucleosome. In addition, as ubH2A has been found to cooperate with many different factors, including linker histone H1, RSF1, PRC, and the FACT complex,[9,18,19] it will be of great interest to investigate how ubH2A correlates with these different factors to perform specific biological functions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/jacs.9b12448.

Supplementary figures and materials and methods (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Ping Chen − *School of Basic Medical Sciences, Advanced Innovation Center for Human Brain Protection, Capital Medical University, Beijing 100054, China; National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China;* orcid.org/0000-0003-0295-0846; Email: chenping@ccmu.edu.cn

Wei Li − *National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China; Songshan Lake Materials Laboratory, Dongguan, Guangdong 523808, China;* orcid.org/0000-0002-2493-1083; Email: weili007@iphy.ac.cn

Authors

Xue Xiao − *National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China; University of Chinese Academy of Sciences, Beijing 100049, China*

Cuifang Liu − *National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China*

Yingxin Pei − *National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China; University of Chinese Academy of Sciences, Beijing 100049, China*

Yi-Zhou Wang − *Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Synthetic Biology Center, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, Guangdong 518124, China*

Jingwei Kong − *National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China; University of Chinese Academy of Sciences, Beijing 100049, China*

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Ke Lu − *National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China; University of Chinese Academy of Sciences, Beijing 100049, China*

Lu Ma − *National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China*

Shuo-Xing Dou − *National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China; University of Chinese Academy of Sciences, Beijing 100049, China;* orcid.org/0000-0002-7201-2081

Peng-Ye Wang − *National Laboratory for Condensed Matter Physics and Key Laboratory of Soft Matter Physics, Institute of Physics, Chinese Academy of Sciences, Beijing 100190, China; University of Chinese Academy of Sciences, Beijing 100049, China; Songshan Lake Materials Laboratory, Dongguan, Guangdong 523808, China*

Guohong Li − *National Laboratory of Biomacromolecules, CAS Center for Excellence in Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China; University of Chinese Academy of Sciences, Beijing 100049, China*

Complete contact information is available at: https://pubs.acs.org/10.1021/jacs.9b12448

Author Contributions

[▽]X.X., C.L., and Y.P. contributed equally.

Notes

The authors declare no competing financial interest.

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