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Structural basis of nucleosome dynamics modulation by histone variants H2A.B and H2A.Z.2.2

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision 14th Jul 2020

Thank you for submitting your manuscript reporting cryo-EM structures for nucleosomes containing H2A variants H2A.B and H2A.Z.2.2 for consideration by The EMBO Journal. Please also excuse the delay in communicating the decision to you, which was due to a delayed review process on account of the current pandemic. We have now however received three referee reports on your study, which are included below for your information.

As you will see, the reviewers are overall positive and express an interest in the study. Nonetheless they also raise some concerns that would need to be addressed in a revised manuscript. In particular, please show and discuss the binding of the PARP1 DBD to the nucleosome (ref #1- point 1; ref #3- point 2, 3) and add the requested additional information for H2A sequence and structure (ref #1- point 5; ref #2- minor points 6, 7). In addition, all referees found that the approach, in particular the use of "precrosslinking" needs to be described in further detail. Referee #2 furthermore raises concerns regarding potential structural artefacts, and proposes further controls for the MNase digest assays (ref #2- points 1, 2), that should be considered. In addition, please carefully consider all other points the referees raise and revise the manuscript accordingly, as well as responding to their comments.

Please note that it is our policy to allow only a single round of major revision. We realize that lab work worldwide is currently affected by the COVID-19/SARS-CoV-2 pandemic and that an experimental revision may be delayed. We can extend the revision time when needed, and we have extended our 'scooping protection policy' to cover the period required for a full revision. However, it is nonetheless important to clarify any questions and concerns at this stage and we encourage you to discuss a revision plan and any potential issues you may foresee as soon as possible.

Referee #1:

This manuscript describes the elucidation of cryo-EM structures containing variants of H2A, namely H2A.B and H2A.Z-2-2 and an analysis of the differences with canonical structures. Especially for H2A.B one of the structures has very nice resolution 2.8 Angstrom one of the higher quality structures, allowing detailed analysis. Both structures compact less DNA than canonical NCPs and a valiant effort is made to detect which differences between H2A and H2A.B are responsible for this, with the conclusion that this all differences contribute to some extent to the shorter DNA compaction. They then focus attention on a short stretch of amino acids 106-129, which they call 'ROF' which seems to have a major effect on the octamer folding. Finally the ROF is also analyzed in H2A.Z.2.2 and shown that also in this context it is important and also affects the SWR1 dependent histone exchange.
Of course this is not the first NCP where this kind of unravelling is seen, but for understanding how they function this work will be important.

The data seem very high quality, the experiments themselves are clearly presented, but nevertheless the manuscript as a whole is somewhat hard to read. This is probably mainly due to the fact that it is not very clear what the 4 differences are that are initially discussed and the ROF, and also why the transition to H2A.Z.2.2 is made. This can probably be changed quite easily by highlighting a bit more the transitions between sections, both in the text and in the title figures.

Other points
- The PARP1 DNA binding domain was used to obtain the high resolution structure and I understand that PARP1 itself is not very well defined, however the location of this domain relative to the nucleosome is surely of interest to quite a few people, if it was not known before. If it was already known it deserves better references, if it wasn't known it deserves a much more extended description and discussion.
- The colours in Fig1 are confusing, as in this figures H2A is orange whereas in all figures H2B is orange
- Line 208 should refer to figure S5c, not figure 5c.
- Please give residue numbers for the 'VAPGED' in the text and refer to the alignment
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- Please include complete data collection information in Table S1. (Detector, voltage,etc.)

Referee #2:

Summary

The authors present a structural and biochemical analysis of nucleosomes containing the H2A variants H2A.B and H2A.z.2.2. As the H2A.B-H2B dimer was unstable, they used a fused construct and performed a pre-crosslinking treatment to prevent dissociation from the NCP, which yielded a resolution of 3.9A by cryoEM. They also screened a number of nucleosome binding domains to use a molecular stabiliser of the H2A.B containing nucleosome, and identified PARP1-DBD, which

yielded a cryoEM structure of 2.8A, albeit without the PARP1-DBD being visible.

Comparison with the canonical H2A nucleosome showed significant disparities, most notably H2A.B wraps only 103 bp of DNA compared to the 147bp of canonical nucleosomes. The lack of DNA wrapping is partly due to a 16 residue truncation within the C-terminal regions, compared to canonical H2A, and also the difference in specific amino acids that mediate interactions with DNA. This is discussed in detail within the MS.

Motif-swapping and mutational analysis were used to investigate at the atomic level the residues/regions that confer the shorter DNA wrapping of H2A.B (Figure 3). Further, the authors characterise the disruptive effect H2A.B has on the histone octamer at high salt, identifying a 'ROF' motif present in the canonical variant, but not H2.B (Figure 4). As H2A.Z.2.2 is another variant that diverges in the ROF motif region, the authors use a number of motif swaps to further demonstrate its importance in octamer stabilisation and MNase protection (Figure 4D, E).

The cryoEM structure of H2A.Z.2.2 is solved to 4.4A resolution and shows only 125 bp of DNA wrapping (Figure 6). The authors then use a dimer exchange assay to show that purified Swr1 from yeast is able to exchange an "H2A.Z.2.2-like" dimer, similarly to H2A.Z.

Overall, I believe this is an important study that gives a structural explanation for a long-standing question in the field, being why does an H2A.Bdb nucleosome wrap less DNA that a normal nucleosome. The experiments appear well performed in most instances, and the manuscript is generally well-written and concise, however I have a number of concerns:

Major concerns:

The obvious concern is that the steps necessary to stabilise the complex (fusion of H2B-H2A.B and pre-fixing), so that a structure can be determined, are actually introducing structural artefacts. Separating the MNase digests (Figure 4B) to base-pair resolution (using a sequencing gel or similar), would give stronger biochemical support for the length of DNA seen in the cryoEM structure.

MNase digestion and octamer folding should also be performed on wild-type H2A.Z.2.2. It seems the authors focused in on the ROF region before H2A.Z.2.2 was introduced in the paper, and so do not use the wild-type as the correct control for mutant comparisons as it should have been.

With regards to the dimer exchange reaction (Figure 6E), wild-type H2A.Z.2.2 should also be used as a control. Ideally, more time points should be implemented to demonstrate the course of the reaction. It seems exchange is complete after the first time point. Additionally, is the difference in time points between the two samples a typographical error - 5 & 30 minutes compared to 15 & 30 minutes?

Minor comments:

Consider the minor correction of the title "Structural mechanism of nucleosome dynamics governed by human histone variants H2A.B and H2A.Z.2.2".

I may have missed it, but I really think some of the seminal papers from the late John Widom's lab, looking at the wrapping and unwrapping of nucleosomes, should be cited within the manuscript.

Especially when using the Widom 601 sequence.

Line 148: "As a control, the globular domain of linker histone H5 (GH5 residues 22-102, designated as GH5-GD) failed to interact H2A.B-NCP." I don't understand the logic behind using H5 globular domain as a control for PARP1-DBD binding. What does it tell you?

Can the mutant constructs in Figure 4 be more clearly linked to the domain diagram (Figure 4A) so that the reader doesn't have to continually go back to the main text/legend? I.e. make it more obvious what each mutant relates to.

Also, the nomenclature describing the mutants can be a little confusing. ">" is usually used to denote and amino acid change, rather than a domain swap. Reading "DD>A" would suggest two aspartic acids replaced with a single alanine, but here it actually means the docking domain replaced with the canonical H2A sequence. Could the authors think of a way to address this?

Figure 5A. Can the secondary structure of H2A be shown in the diagram to help orientate the reader (as in Figure 4A)? Alternatively, all constructs used in the article could be presented together (i.e. combining Figure 4A and Figure 5A).

There is a conserved stretch of 6 aa present in most H2A forms but not in H2A.B, leading the authors to name it the Regulating-Octamer-Folding sequence. Does it truly regulate? Or is it simply structurally necessary?

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In this manuscript, the authors report the atomic resolution cryo-EM structures of the nucleosomes containing human histone variant H2A.B and H2A.Z.2.2. These structures are very different from those of canonical nucleosomes. They identified a six-residue sequence, termed regulating-octamer-folding (ROF), that is responsible for the structural differences. They further demonstrated the role of H2A.Z.2.2 in histone replacement reaction catalyzed by chromatin remodeler Swr1 of budding yeast.

Nearly all of the nucleosome structures we know today contain ~147 DNA and share similar structural features. The structures reported in this manuscript represent rare cases of the nucleosome structures, which are substantially different from the canonical nucleosome structures in both core histones and nucleosomal DNA. These high-quality structures are important for understanding the functions of histone variants and the physical bases of the histone-histone and histone-DNA interactions. In addition, in this study, the authors used a new approach to overcome the dissociation problem of the nucleosome by screening proteins that bind to the nucleosomal DNA, which may be applicable in the studies of nucleosome structures with similar dissociation issues. Overall, the work contributes significantly to the structural studies of nucleosomes and histone variants.

I have some minor issues regarding the clarity of the manuscript.

The authors stated that "To solve this problem, H2A.B-NCP is subject to the pre-crosslinking treatment, which improves both the yield and homogeneity of samples for cryo-EM study (Fig. S1C and S1D)." I suppose the authors meant they performed the cross-linking experiment before the gradient centrifugation that is commonly used in the grad-fix experiment. It is better to describe

"the pre-crosslinking treatment" briefly in the main text.

The authors used the PARP-1 DBD binding domain to stabilize the nucleosome. The rational to choose the PARP-1 DBD and others for screening should be described in the main text.

The authors stated that "Interestingly, the 2.8 Å H2A.B-NCP lacks ~5 bp terminal DNA shown in the 3.9 Å H2A.B-NCP, indicating a dynamic nature of the interaction at the open ends of H2A.B-NCP DNA (Fig. S3)." This interpretation is not clear to me. Could it be caused by the binding of PARP-1 DBD? In any case, the authors may want to describe how PARP-1 DBD binds to the nucleosome in their 6 Å resolution structure.

It's not clear to me whether the authors were able to define the DNA bases and the orientation of the DNA. I suppose they could. But I would like to see they show the density and the model fitting in detail. In particular, they should show that the DNA can only fit the density map well in one direction but not in the opposite direction.

Referee' Comments:

Referee #1:

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We have modified the text, including the paragraph subtitle and the figure title as suggested (page 8). We thank the reviewer for the suggestion.

Other points

- The PARP1 DNA binding domain was used to obtain the high resolution structure and I understand that PARP1 itself is not very well defined, however the location of this domain relative to the nucleosome is surely of interest to quite a few people, if it was not known before. If it was already known it deserves better references, if it wasn't known it deserves a much more extended description and discussion.

We thank the reviewer for the suggestion to improve the manuscript. The interaction between PARP1-DBD and H2A.B-NCP has been discussed as follows "PARP1 is a highly abundant and ubiquitous chromatin-associated enzyme in nucleus. PARP1-DBD contains three Zinc finger domains (Zn1, Zn2, Zn3) that can all bind to DNA (Kim *et al*, 2004; Muthurajan *et al*, 2014) and confer PARP1 the ability to recognize a diverse range of DNA breaks and atypical DNA secondary structures (Alemasova & Lavrik, 2019). Structural classifications of Pb-H2A.B-NCP reveal one dataset that can be reconstructed to 6.2 Å resolution, wherein the PARP1-DBD was found to interact with both the out wrap and inner wrap of DNA (Fig EV2F). The low resolution precludes the determination of binding details

for PARP1-DBD. Notably, whether this binding mode is adopted by full length PARP1 remains unknown. Further studies are needed to answer this question" (page 11, line 443-453).

- The colours in Fig1 are confusing, as in this figures H2A is orange whereas in all figures H2B is orange

We recolored H2A.B in orange in all figures. In contrast, H2A is colored in yellow wherever H2A.B and H2A are both presented for structure comparison. Moreover, H2B are colored in red and magenta, respectively, in case two H2B are shown for structural comparison.

- Line 208 should refer to figure S5c, not figure 5c.

The error has been corrected. We thank the reviewer for pointing it out.

- Please give residue numbers for the 'VAPGED' in the text and refer to the alignment

We have modified the text and referred the residues to the alignment (page 6, line 221).

- Please give at least one alignment of full length H2A, can be either in figure 4 or 8c, and please number all sequences with their own numbering scheme (at least at the start of each line, it is an option in ESPRIPT)

We have modified the text and alignment accordingly (Appendix Fig S3).

- Please include complete data collection information in Table S1. (Detector, voltage,etc.)
Table 1 has been modified as suggested.

Referee #2:

Summary

The authors present a structural and biochemical analysis of nucleosomes containing the H2A variants H2A.B and H2A.z.2.2. As the H2A.B-H2B dimer was unstable, they used a fused construct and performed a pre-crosslinking treatment to prevent dissociation from the NCP, which yielded a resolution of 3.9A by cryoEM. They also screened a number of nucleosome binding domains to use a molecular stabiliser of the H2A.B containing nucleosome, and identified PARP1-DBD, which yielded a cryoEM structure of 2.8A, albeit without the PARP1-DBD being visible.

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authors characterise the disruptive effect H2A.B has on the histone octamer at high salt, identifying a 'ROF' motif present in the canonical variant, but not H2.B (Figure 4). As H2A.Z.2.2 is another variant that diverges in the ROF motif region, the authors use a number of motif swaps to further demonstrate its importance in octamer stabilisation and MNase protection (Figure 4D, E).

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We thank the reviewer for the positive comments.

Major concerns:

The obvious concern is that the steps necessary to stabilise the complex (fusion of H2B-H2A.B and pre-fixing), so that a structure can be determined, are actually introducing structural artefacts. Separating the MNase digests (Figure 4B) to base-pair resolution (using a sequencing gel or similar), would give stronger biochemical support for the length of DNA seen in the cryoEM structure.

A number of previous studies using linked H2A-H2B dimer or linked H2A.Z-H2B dimer have demonstrated that the linker does not altered the structure of regular dimers no matter the dimers is in the nucleosome-free form or nucleosome-embedded form (Hong *et al*, 2014; Hu *et al*, 2017; Liang *et al*, 2016; Wang *et al*, 2019, 1). Apart from that, we and others showed that the fusion histone dimers do not affect nucleosome assembly (Hu *et al*, 2017 and this study). Moreover, the crystal structure of linked H2A.B-H2B dimer resembles the cryo-EM structure of their counterparts in H2A.B-NCP (Dai *et al*, 2018). All these results suggested that the possibility of H2A-H2A.B fusion causing structural artefacts is low.

The term "pre-crosslinking" refers to the crosslinking experiment before the gradient centrifugation, which is commonly used in the cryo-EM sample preparation. While the samples used for determining the 3.9 Å H2A.B-NCP structure are subject to crosslinking treatment, samples used for determining the 2.8 Å H2A.B-NCP structure are prepared using the grafix approach. Indeed, the structures of these two samples are very similar, suggesting the "pre-crosslinking" treatment unlikely causes structural artefacts. Nonetheless, to avoid using inaccurate term of "pre-crosslinking" in the revised manuscript, we modified the manuscript as follows, "To solve this problem, we performed the crosslinking experiment before the gradient centrifugation, which is commonly used in the cryo-EM sample preparation. This treatment improves the yield and homogeneity of H2A.B-NCP samples for cryo-EM study (Fig EV1C and D)" (page 4, line 130-133). We thank the reviewer for the suggestion to improve the manuscript.

We agree with the reviewer that the measurement of the length of DNA is important. We measure the length of H2A.B-NCP DNA protected against MNase digestion using a similar strategy used by (Bönisch *et al*, 2012). To achieve this goal, we extracted DNA treated by MNase and analyzed it by 10% Native-PAGE. A standard curve is prepared by plotting the length of each DNA standard versus the mobility measured from the EMSA. As such, the length of the 103 bp DNA is calculated from the standard curve (Fig EV3).

MNase digestion and octamer folding should also be performed on wild-type H2A.Z.2.2. It seems the authors focused in on the ROF region before H2A.Z.2.2 was introduced in the paper, and so do not use the wild-type as the correct control for mutant comparisons as it should have been.

We thank the reviewer for the suggestion to improve the manuscript. We have shown that the wild-type H2A.Z.2.2 prevents octamer folding and reduces the resistance of Z.2.2-NCP against MNase digestion (Fig EV5E and F). We supplemented the text with these results (Page 8 line 323 and Page 9 line 354).

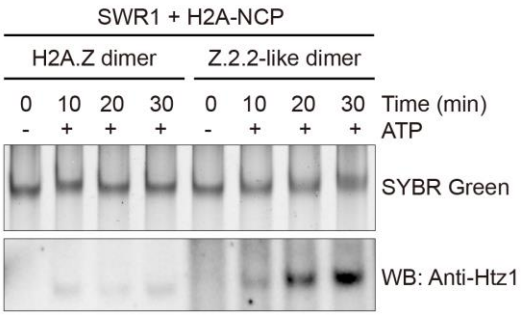
With regards to the dimer exchange reaction (Figure 6E), wild-type H2A.Z.2.2 should also be used as a control. Ideally, more time points should be implemented to demonstrate the course of the reaction. It seems exchange is complete after the first time point. Additionally, is the difference in time points between the two samples a typographical error - 5 & 30 minutes compared to 15 & 30 minutes?

H2A.Z.2.2 is a primate-specific histone variant that is supposed to be incorporated into chromatin by remodeler SRCAP/P400. Ideally, one should use human SRCAP/P400 and human H2A.Z.2.2 to examine the effect of H2A.Z.2.2 on histone replacement. But the preparation of human SRCAP/P400 has been demonstrated technically challenging. To overcome this difficulty, we generated a yeast Z.2.2-like mutant and investigated how this mutant influences the SWR1-catalyzed histone replacement. We avoided using human H2A.Z.2.2 and H2A.Z in the assay because yeast SWR1 failed to replace H2A-NCP with human H2A.Z at our experimental condition.

To show the course of histone replacement reaction, we repeated the SWR1-catalyzed histone replacement show in Fig 6E and demonstrated that the exchange rate of Z.2.2-like at 15-min is indeed less than the exchange rate at 30-min (Fig. 6E). We performed additional experiments to examine the Z.2.2 exchange rate at 10-min and found incompleted exchange as well (Fig. R1). We thank the reviewer for the suggestion to improve the manuscript.

The typographical error has been corrected in the revised version.

Fig. R1



Minor comments:

Consider the minor correction of the title “Structural mechanism of nucleosome dynamics governed by human histone variants H2A.B and H2A.Z.2.2”.

The title is modified in the revised version. We thank the reviewer for the suggestion to improve the manuscript.

I may have missed it, but I really think some of the seminal papers from the late John Widom’s lab, looking at the wrapping and unwrapping of nucleosomes, should be cited within the manuscript. Especially when using the Widom 601 sequence.

We have cited John Widom’s work on 601 sequence in the manuscript (line 126).

Line 148: “As a control, the globular domain of linker histone H5 (GH5 residues 22-102, designated as GH5-GD) failed to interact H2A.B-NCP.” I don’t understand the logic behind using H5 globular domain as a control for PARP1-DBD binding. What does it tell you?

PARP1 and linker histone H1 compete for nucleosome binding in vitro and exhibit a reciprocal pattern of the chromatin binding (Kim *et al*, 2004; Shukla *et al*, 2011). As PAPR1-DBD has a demonstrated ability to interact with H2A.B-NCP, we asked whether the linker histone can bind to H2A.B-NCP as well. However, the globular domain of linker histone H5 (GH5 residues 22-102, designated as GH5-GD) failed to interact H2A.B-NCP, suggesting the binding is PAPR1-DBD specific. The manuscript has been modified accordingly (page 4, line 149-155). We thank the reviewer for the suggestion to improve the revisions.

Can the mutant constructs in Figure 4 be more clearly linked to the domain diagram (Figure 4A) so that the reader doesn’t have to continually go back to the main text/legend? I.e. make it more obvious what each mutant relates to.

Also, the nomenclature describing the mutants can be a little confusing. “>” is usually used to denote and amino acid change, rather than a domain swap. Reading “DD>A” would suggest two aspartic acids replaced with a single alanine, but here it actually means the docking domain replaced with the canonical H2A sequence. Could the authors think of a way to address this?

We modified Figure 4A by recoloring H2A/H2A.B and showing the mutants in the domain diagram. As the reviewer suggested, the H2A.B mutant of docking domain (DD) swapping is denoted as "H2A.B-DD^{H2A}" rather than "DD>A." This rule has been applied to the nomenclature describing the mutants in the revised manuscript. We thank the reviewer for the suggestion.

Figure 5A. Can the secondary structure of H2A be shown in the diagram to help orientate the reader (as in Figure 4A)? Alternatively, all constructs used in the article could be presented together (i.e. combining Figure 4A and Figure 5A).

We modified the Figure 5A by showing the secondary structure of H2A in the diagram.

There is a conserved stretch of 6 aa present in most H2A forms but not in H2A.B, leading the authors to name it the Regulating-Octamer-Folding sequence. Does it truly regulate? Or is it simply structurally necessary?

In this study, we define the ROF as a stretch of amino acid shown in all H2A. Hence, both canonical H2A and H2A variants contain ROF. The ROF of canonical H2A, as pointed out by the reviewer, is required for octamer folding and nucleosome structure integrity. However, the ROF of H2A.B or H2A.Z.2.2 leads to defects of octamer folding and nucleosome structure, suggesting the ROF in different H2A have different outcomes. As such, we named it the Regulating-Octamer-Folding sequence.

Referee #3:

In this manuscript, the authors report the atomic resolution cryo-EM structures of the nucleosomes containing human histone variant H2A.B and H2A.Z.2.2. These structures are very different from those of canonical nucleosomes. They identified a six-residue sequence, termed regulating-octamer-folding (ROF), that is responsible for the structural differences. They further demonstrated the role of H2A.Z.2.2 in histone replacement reaction catalyzed by chromatin remodeler Swr1 of budding yeast.

Nearly all of the nucleosome structures we know today contain ~147 DNA and share similar structural features. The structures reported in this manuscript represent rare cases of the nucleosome structures, which are substantially different from the canonical nucleosome structures in both core histones and nucleosomal DNA. These high-quality structures are important for understanding the functions of histone variants and the physical bases of the histone-histone and histone-DNA interactions. In addition, in this study, the authors used a new approach to overcome the dissociation problem of the nucleosome by screening proteins that bind to the nucleosomal DNA, which may be applicable in the studies of nucleosome structures with similar dissociation issues. Overall, the work contributes significantly to the structural studies of nucleosomes and histone variants.

We thank the reviewer for the positive comments.

I have some minor issues regarding the clarity of the manuscript.

The authors stated that “To solve this problem, H2A.B-NCP is subject to the pre-crosslinking treatment, which improves both the yield and homogeneity of samples for cryo-EM study (Fig. S1C and S1D).” I suppose the authors meant they performed the crosslinking experiment before the gradient centrifugation that is commonly used in the grad-fix experiment. It is better to describe “the pre-crosslinking treatment” briefly in the main text.

We are sorry for the inaccurate statement and thank the reviewer for the suggestion. The manuscript has been modified accordingly. “To solve this problem, we performed the crosslinking experiment before the gradient centrifugation, which is commonly used in the cryo-EM sample preparation. This treatment improves the yield and homogeneity of H2A.B-NCP samples for cryo-EM study.” (page 4, line 130-133) Please see our response to Referee #1.

The authors used the PARP-1 DBD binding domain to stabilize the nucleosome. The rational to choose the PARP-1 DBD and others for screening should be described in the main text.

We thank the reviewer for the suggestion to improve the manuscript. The rationale is explained as follows “A previous study using a single-chain antibody fragment (scFv) recognizing nucleosomes can successfully stabilize nucleosome and achieve the atomic resolution nucleosome cryo-EM structures (Zhou *et al*, 2019). In light of this finding, we predicted that non-histone proteins with demonstrated nucleosome binding ability might stabilize H2A.B-NCP by attenuating nucleosome opening.” (page 4, line 140-154)

The authors stated that “Interestingly, the 2.8 Å H2A.B-NCP lacks ~5 bp terminal DNA shown in the 3.9 Å H2A.B-NCP, indicating a dynamic nature of the interaction at the open ends of H2A.B-NCP DNA (Fig. S3).” This interpretation is not clear to me. Could it be caused by the binding of PARP-1 DBD? In any case, the authors may want to describe how PARP-1 DBD binds to the nucleosome in their 6 Å resolution structure.

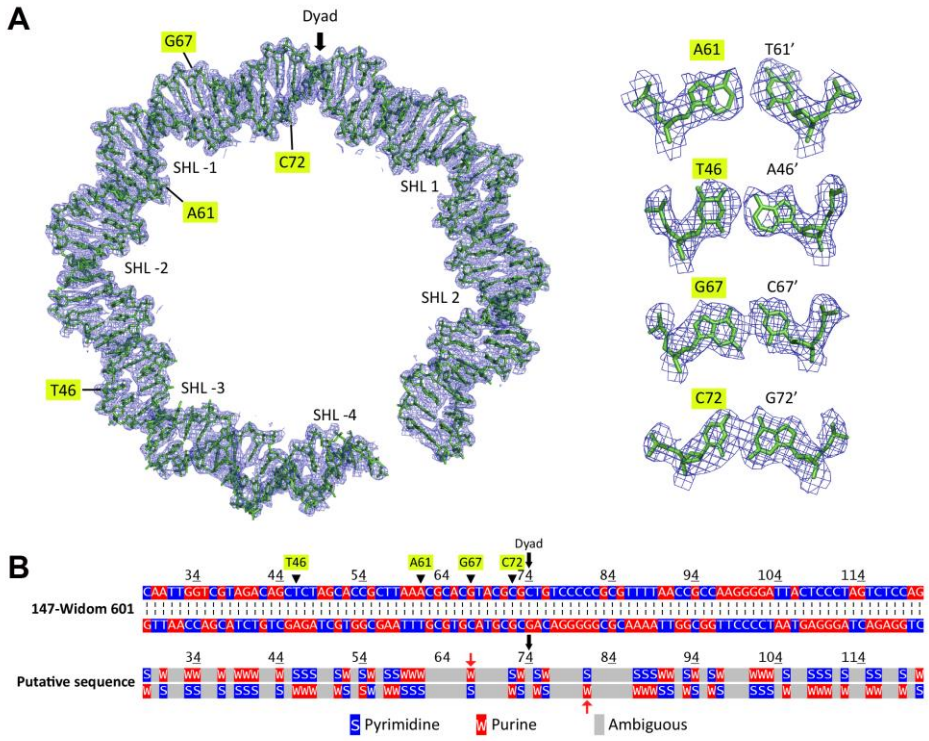
We agree with the reviewer on this point. Although the absence of additional ~5 bp DNA in the 2.8 Å H2A.B-NCP structure suggested that this portion of DNA is more flexible as compared to other components, we could not rule out the possibility that the flexibility may be caused by PAPR1-DBD binding. Therefore, we modify the manuscript as follows “the 2.8 Å H2A.B-NCP lacks ~5 bp terminal DNA shown in the 3.9 Å H2A.B-NCP, indicating this part of DNA is flexible.” (line 166-167)

Moreover, we discussed the interaction between PARP1-DBD and H2A.B-NCP in the revised manuscript (page 11, line 443-457). Please see our response to Referee #1. We thank the reviewer for the suggestion to improve the manuscript.

It’s not clear to me whether the authors were able to define the DNA bases and the orientation of the DNA. I suppose they could. But I would like to see they show the density and the model fitting in detail. In particular, they should show that the DNA can only fit the density map well in one direction but not in the opposite direction.

We thank the reviewer for the suggestion to improve the manuscript. Indeed, the 2.8 Å H2A.B-NCP structure applied C2 symmetry (dyad) allowed us to discriminate the pyrimidines (C and T) from those of purines (A and G) (Fig. R2A). In the structure, the density map of the symmetry-related nucleotide pairs will be averaged out. Therefore, one could distinguish the cryo-EM density of the paired pyrimidines from those of the paired purine, as the single-ring structure of pyrimidines is smaller than the two-ring structures of purines (Fig. R2A). In contrast, the cryo-EM density of the nucleotide pairs containing one pyrimidine and one purine is ambiguous, as one can not tell which nucleotide is pyrimidine or purine. This rule allowed us to assign multiple pyrimidines and purines and generate a ~93 bp of DNA with a putative sequence (Fig. R2B). Importantly, the pyrimidine/purine pattern in the putative DNA sequence exclusively matches the pyrimidine/purine pattern of the Widom 601 DNA ranging from 28 bp to 122 bp, suggesting the central region of Widom 601 DNA wraps around the histone octamer (Fig. R2B). The red arrows in Fig. R2B indicate the symmetry-related nucleotide pairs that contain two purines. The cryo-EM density of the paired purines is compatible with the two ring structure of purines (G67 in Fig. R2A)

Fig. R2



Example density of the DNA in Pb-H2A.B-NCP.

A. Cryo-EM density map of the local nucleosomal DNA fitted with the refined atomic model. The nucleosome dyad and superhelical turns of H2A.B nucleosomal DNA at superhelical locations SHL -4 through SHL +2 are indicated. Density of representative nucleotides T46, A61, G67, C72 are shown in the right pane. The density of pyrimidine (C and T) can be distinguished from that of purine (A and G).

B. Schematic view of the center region of 147-bp Widom 601 sequence and the putative sequence of H2A.B-NCP DNA. The pyrimidines (C and T) and purines (A and G) are colored in blue and red, respectively. The pyrimidines (S) and purines (W) in the H2A.B-NCP DNA are determined based on the nucleotide density. In the structure, the density map of two nucleotides disposed symmetrically are averaged out. If the paired nucleotides (centered on the dyad) are pyrimidines (or both purines), the cryo-EM density of pyrimidines and purines could be distinguished, given that the single-ring structure of pyrimidines is smaller than the two-ring structures of purines. However, if a pair of symmetry-related nucleotides contains one pyrimidine and one purine, the averaged density will be too ambiguous to tell which one is pyrimidine (or purine). This rule allows us to assign some pyrimidine and purine, generating a ~93 bp of DNA with a putative sequence. Importantly, the pyrimidine (or purine) pattern in the putative DNA sequence exclusively matches the pyrimidine/purine pattern of the Widom 601 DNA ranging from 28 bp to 122 bp, suggesting that the center region of Widom 601 DNA wraps around the histone octamer.

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2nd Editorial Decision 11th Sep 2020

Thank you for submitting your revised manuscript, we have now received the reports from the three initial referees (see comments below). I am pleased to say that they overall find that their comments have been satisfactorily addressed and now support publication. Referee #2 makes a suggestion regarding Fig. 6E, which you may want to consider for the final revised version. In this version, I would like to ask you to also address a number of editorial issues that are listed in detail below. Please make any changes to the manuscript text in the attached document only using the "track changes" option. Once these remaining issues are resolved, we will be happy to formally accept the manuscript for publication.

REFEREE REPORTS

Referee #1:

The authors have addressed all the points of the reviewers satisfactorily;
It would be good to check the spelling of PARP throughout the manuscript, as it is currently misspelled at least twice (PAPR)

Referee #2:

The authors have addressed all of our concerns comprehensively. Additional control experiments have been carried out and included in the revised version, all of which confirm the authors original conclusions. We suggest the manuscript to be published.

One minor comment. The authors have included a repeat experiment regarding Swr1 dimer exchange in the rebuttal (Fig. "R1"). It strikes me that the repeat is actually than the one original presented in Figure 6E (as it contains more time points from the reaction). They may like to consider either swapping it out, or including Fig "R1" in the Appendix/EV Figures as a demonstration of reproducibility.

Referee #3:

The revised manuscript has addressed my concerns. I recommend its publication.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Zheng Zhou
Journal Submitted to: The EMBO journal
Manuscript Number: EMBOJ-2020-105907R

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be iustified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	NA
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C- Reagents

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