EMBO reports

CENP-C unwraps the human CENP-A nucleosome through the H2A C-terminal tail

Ahmad Ali-Ahmad, Silvija Bilokapić, Ingmar B. Schäfer, Mario Halić, Nikolina Sekulić

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Revision received:	31st Jul 2019
Editorial Decision:	5 August 2019
Revision received:	7 August 2019
Accepted:	8 August 2019

Editor: Esther Schnapp

Transaction Report:

Please note that the manuscript was previously reviewed at another journal and the reports were taken into account in the decision making process at EMBO Reports. Since the original reviews, which were submitted to EMBO Reports, are not subject to EMBO Press' transparent review process policy, the reports and author response cannot be published.

1st Editorial Decision 24th Jul 2019

Thank you for the submission of your revised manuscript to EMBO reports. I have now heard back from the advisor who was asked to assess it (the comments are pasted below). I am happy to tell you that s/he supports the publication of your study pending minor revisions. Please address these last concerns in the final manuscript.

A few other changes will also be required:

With 4 main figures the manuscript should be layed out as a short report with combined results and discussion sections and not more than 27.000 characters including spaces but excluding materials and methods and references. If you prefer to keep the results and discussion separate, you need to have 6 or more main figures in the main manuscript files.

Please send us a completed author checklist, which you can download from our author guidelines <https://www.embopress.org/page/journal/14693178/authorguide>. Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF (see below).

As co-corresponding author, Mario Hallic needs to add his ORCID number to his personal profile page in our online manuscript submission system.

Please upload all Figures as individual, high-resolution figure files. The Figure legends need to be added to the main manuscript file, following the References.

Fig. 3F and 4F are not called out in the manuscript text, please correct.

Please send us the main manuscript as a word file, including the figure legends and if applicable the

legends for EV figures.

The paper's contributing authors need to be entered in our online manuscript tracking system.

The Materials & Methods need to be removed from the Supplemental materials, and need to be added to the main manuscript, after the Discussion.

Acknowledgements, Conflict of interest and Author contributions need to be moved to after M&M and before the References.

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You can either rename the Suppl Material to "Appendix" (correct nomenclature is "Appendix Figure S1" and "Appendix Table S1") with a Table of content that should mention the figures and tables and page numbers. Alternatively, you can have up to 6 "Expanded view" (EV) figures that are collapsible/expandable online. EV Figures should be cited as 'Figure EV1, Figure EV2" etc in the text and their respective legends should be included in the main text after the legends of the main figures. See detailed instructions regarding expanded view here:
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Several gels in Fig 2 B,C,E are spliced together. The splices need to be clearly indicated by white space and/or a black line. It would be better to submit the source data for these gels so that it is clear where the bands come from. Source data gel bands and weight markers need to be labeled. The source data will be linked online to their main figures. Additional information on source data and instruction on how to label the files are available at
<https://www.embopress.org/page/journal/14693178/authorguide#sourcedata>.

Figures S2A,B and S5A,B need scale bars.

The gels shown in Fig 3 are over-exposed. Please send us better images if possible.

Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database (see
https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). The accession numbers and databases should be listed in a formal "Data Availability" section placed after Materials & Method (see also
https://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please note that the Data Availability Section is restricted to new primary data that are part of this study.
* Note - All links should resolve to a page where the data can be accessed. *

No information on funding is provided.

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We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

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File is available with this article, as the authors have chosen not to make the review process public in this case."

I look forward to seeing a final version of your manuscript as soon as possible. Please let me know if you have questions or comments regarding the revision.

Kind regards,
Esther

Esther Schnapp, PhD
Senior Editor
EMBO reports

Referee #1:

For this study, Ali-Ahmad, Sekulic, and colleagues conduct structural and biochemical studies to analyze the interaction between CENP-A and CENP-C. Recent work has also provided structural and functional insights into the nature of the CENP-A-CENP-C interaction and the individual structure of the CENP-A nucleosome. However, this paper goes substantially beyond prior work to provide important new insights into this interaction and these individual proteins. Given the central importance of these proteins to epigenetically marking the centromere and providing the platform for kinetochore assembly, the combination of the data in this paper provides an important advance that should be of interest to both centromere and chromatin biologists.

In particular, the authors provide:
1) High quality structures of the CENP-A nucleosome and CENP-A bound to a fragment of CENP-C, allowing them to detect the basis for this interaction and the changes to the CENP-A nucleosome.
2) Advances in understanding the flexible interaction of CENP-A with DNA, which results in unwrapping. This includes information in their structure, MNase digestion experiments on different DNA substrates, and chimeric experiments with H3 to implicate the CENP-A tail in this process.
3) Important information on the relative binding and affinity of the different CENP-C interaction motifs that have been proposed for CENP-A. This includes generating additional mutants and truncations to precisely test these interaction domains, as well as testing their binding to H3 nucleosomes, where the data clearly demonstrates that they lack specificity for histones generally (i.e., only bind CENP-A).

Each of these (and additional data in the paper) has important structural and functional implications.

Based on the point-by-point response, the authors have made a large number of additional changes since the prior rounds of review. These new experiments significantly add to the paper, and appear to have clearly addressed the vast majority of the technical and conceptual points. As such, I personally feel that they have more than met the bar for publications in EMBO Reports. The technical quality of this paper is very high, and the combined experiments do make an important scientific advance.

My remaining concerns are exclusively textual. This paper is clear overall, but the writing has extensive grammatical issues throughout the entire paper. This seems beyond the standard changes that would occur during copy editing, and in its present form make the paper complex to read at various places. This manuscript needs to be received and edited to address these issues, and ideally make the paper more accessible overall. These wording issues also may explain in part why the previous reviewers were not as enthusiastic about the paper as they should have been. For example, I would also suggest that the authors revise their abstract. Their point-by-point response to the previous reviewer #1 (first major response) beautifully articulates some of the key advances in this paper and why it deserves to be in a major journal. However, some of this gets lost in the abstract. Rewording the abstract to more clearly highlight the key conceptual advances would be helpful. Similarly, the title is rather descriptive. This is probably fine as is, but it could be beneficial to focus on the advances related to the CENP-A-C interaction instead of simply stating that they solved a structure.

1st Revision - authors' response 31st Jul 2019

We have now completed requested changes and uploaded manuscript as a text file and all of the figures as high-res pdf files in EMBO Reports on-line system.

Please let me know if there is anything else I need to do.

_____

Point-by-point response on revisions requested:

1. With 4 main figures the manuscript should be layed out as a short report with combined results and discussion sections and not more than 27.000 characters including spaces but excluding materials and methods and references. If you prefer to keep the results and discussion separate, you need to have 6 or more main figures in the main manuscript files.

We now have 6 main figures.

2. Please send us a completed author checklist, which you can download from our author guidelines <https://www.embopress.org/page/journal/14693178/authorguide><https://www.embopress.org/page/journal/14693178/authorguide%3E>;. Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF (see below).

Included.

3. As co-corresponding author, Mario Hallic needs to add his ORCID number to his personal profile page in our online manuscript submission system.

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5. Fig. 3F and 4F are not called out in the manuscript text, please correct.

Corrected.

6. Please send us the main manuscript as a word file, including the figure legends and if applicable the legends for EV figures.

Done.

7. The paper's contributing authors need to be entered in our online manuscript tracking system.

Done.

8. The Materials & Methods need to be removed from the Supplemental materials, and need to be added to the main manuscript, after the Discussion.

Done.

9. Acknowledgements, Conflict of interest and Author contributions need to be moved to after M&M and before the References.

Done.

10. The REFERENCE FORMAT needs to be correct from alphabetical to numerical. The EMBO reports format is also in EndNote (up to 10 authors listed before "et al")

Done.

11. You can either rename the Suppl Material to "Appendix" (correct nomenclature is "Appendix Figure S1" and "Appendix Table S1") with a Table of content that should mention the figures and tables and page numbers. Alternatively, you can have up to 6 "Expanded view" (EV) figures that are collapsible/expandable online. EV Figures should be cited as 'Figure EV1, Figure EV2" etc in the text and their respective legends should be included in the main text after the legends of the main figures. See detailed instructions regarding expanded view here: <https://www.embopress.org/page/journal/14693178/authorguide#expandedview><https://www.embopress.org/page/journal/14693178/authorguide#expandedview%3E>;

We have 6 EV Figures and 2 Appendix tables.

12. Several gels in Fig 2 B,C,E are spliced together. The splices need to be clearly indicated by white space and/or a black line. It would be better to submit the source data for these gels so that it is clear where the bands come from. Source data gel bands and weight markers need to be labeled. The source data will be linked online to their main figures. Additional information on source data and instruction on how to label the files are available at <https://www.embopress.org/page/journal/14693178/authorguide#sourcedata><https://www.embopress.org/page/journal/14693178/authorguide#sourcedata%3E>;.

Done.

13. Figures S2A,B and S5A,B need scale bars.

Done.

14. The gels shown in Fig 3 are over-exposed. Please send us better images if possible.

Corrected.

15. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database (seehttps://www.embopress.org/page/journal/14693178/authorguide#datadeposition). The accession numbers and databases should be listed in a formal "Data Availability" section placed after Materials & Method (see alsohttps://www.embopress.org/page/journal/14693178/authorguide#datadeposition). Please note that the Data Availability Section is restricted to new primary data that are part of this study.
* Note - All links should resolve to a page where the data can be accessed. *

We have deposited pdbs and cryoEM maps and we have included accession codes in our submission.

16. No information on funding is provided.

This is provided in the acknowledgement section. Is this OK?

17. EMBO press papers are accompanied online by A) a short (1-2 sentences) summary of the findings and their significance, B) 2-3 bullet points highlighting key results and C) a synopsis image that is 550x200-400 pixels large (the height is variable). You can either show a model or key data in the synopsis image. Please note that text needs to be readable at the final size. Please send us this information along with the revised manuscript.

Text is provided with the manuscript and image is sent as a separate file.

18. We would also welcome the submission of cover suggestions, or motifs to be used by our Graphics Illustrator in designing a cover.

We have an idea for the cover page based on an image from shutterstock. I would be happy to work on it with Graphics Illustrator. Alternatively, we can provide a processed illustration after 15.08.

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We support publishing of the Review Process File.

I look forward to seeing a final version of your manuscript as soon as possible. Please let me know if you have questions or comments regarding the revision.

2nd Editorial Decision 5 August 2019

Thank you for sending the revised manuscript files. However, not all points have been addressed.

It looks like the tables appear twice now. Please either name the tables Table EV1 and Table EV2 and upload them as individual files, OR include them in the Appendix file in which case they should be named Appendix Table S1, etc. The Appendix needs a table of content with page numbers. Please move the methods in the Appendix to the main manuscript file. If the methods only refer to Appendix figures they can stay in the Appendix file.

I overlooked that the manuscript has both EV figures and an Appendix file. In this case, only 5 EV figures can be included. Please move one EV figure to the Appendix file. And please correct all callouts in the manuscript text.

I attach to this email a word file with comments on the figure legends (and may be some more). Please address all of these comments and send us back a corrected word file.

The data availability section is missing the accession IDs and the URLs. Both must be provided in order for us to proceed with your manuscript.

Fig 4 has no 'F' panel.
Fig 1F is labelled as 1D in the legend.
Figs EV2B + EV5B still need scale bars.

I look forward to seeing a newly revised manuscript as soon as possible.

2nd Revision - authors' response 7 August 2019

I hope that I have addressed all of requested changes.

I have included urls for all of the data but some of it might become active only later today.

I am also enclosing word document of the manuscript with markups for easier follow up of the changes I have introduced.

Please let me know if there is anything else I need to do.

3rd Editorial Decision 8 August 2019

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal. I noticed that the title and abstract do not mention from which species the CENP-A structure is. I would like to suggest to add this information, both to the title and abstract (if I remember correctly you provide the human structure). You can send me the new title and abstract by email and we will replace the current text for you. Thank you.

At the end of this email I include important information about how to proceed. Please ensure that you take the time to read the information and complete and return the necessary forms to allow us to publish your manuscript as quickly as possible.

As part of the EMBO publication's Transparent Editorial Process, EMBO reports publishes online a Review Process File to accompany accepted manuscripts. As you are aware, this File will be published in conjunction with your paper and will include the referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript.

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Corresponding Author Name: Nikolina Sekulic
Journal Submitted to: EMBO Reports
Manuscript Number: EMBOR-2019-48913

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 justified
- 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 $\chi 2$ 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

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1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	N/A
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	N/A
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	N/A
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	N/A
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4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	N/A
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Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Mnase experiments were done in triplicates. Standar deviation (mean of repeated measurements of 3 independently prepared samples) is shown in all main figures.

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Is there an estimate of variation within each group of data?	N/A
Is the variance similar between the groups that are being statistically compared?	N/A

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	N/A
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N/A

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	N/A
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	N/A

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
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F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	Provided
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