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A Dual Role for the Chromatin Reader ORCA/LRWD1 in Targeting the Origin Recognition Complex to Chromatin

Sumon Sahu, Babatunde Ekundayo, Ashish Kumar, and Franziska Bleichert
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(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.)

Dr. Franziska Bleichert
Yale University
Molecular Biophysics and Biochemistry
260 Whitney Avenue
YSB 345
New Haven, CT 06520

12th Jul 2023

Re: EMBOJ-2023-114654
A Dual Role for the Chromatin Reader ORCA/LRWD1 in Targeting the Origin Recognition Complex to Chromatin

Dear Franziska,

Thank you for submitting your study on ORCA/LRWD1 to The EMBO Journal. Three referees with expertise in structural (#1) and functional (#2, #3) analyses of eukaryotic replication have now evaluated it. As you will see from their reports copied below, they all consider the findings of the study of interest and importance, and also appreciate the technical quality of the work. After incorporating a number of specific/minor points raised in the reviews, we shall therefore be happy to offer publication of a revised version in our journal.

I should remind you that it is our policy to allow only a single round of (major) revision, making it important to carefully respond to all points raised at this stage; therefore, please do not hesitate to contact me in case you would like to discuss any of the issues raised by the reviewers. Also, should revision require more time than our default three-months revision period, we would be open to offering an extension, during which our 'scooping protection' (meaning that competing work appearing elsewhere in the meantime will not affect our considerations of your study) would of course remain valid.

Detailed information on preparing, formatting and uploading a revised manuscript can be found below and in our Guide to Authors. Thank you again for the opportunity to consider this work for The EMBO Journal, and I look forward to your revision!

With kind regards,

Hartmut

Hartmut Vodermaier, PhD
Senior Editor, The EMBO Journal
h.vodermaier@embojournal.org

*** PLEASE NOTE: All revised manuscript are subject to initial checks for completeness and adherence to our formatting guidelines. Revisions may be returned to the authors and delayed in their editorial re-evaluation if they fail to comply to the following requirements (see also our Guide to Authors for further information):

1) Every manuscript requires a Data Availability section (even if only stating that no deposited datasets are included). Primary datasets or computer code produced in the current study have to be deposited in appropriate public repositories prior to resubmission, and reviewer access details provided in case that public access is not yet allowed. Further information: embopress.org/page/journal/14602075/authorguide#dataavailability

2) Each figure legend must specify
- size of the scale bars that are mandatory for all micrograph panels
- the statistical test used to generate error bars and P-values
- the type error bars (e.g., S.E.M., S.D.)
- the number (n) and nature (biological or technical replicate) of independent experiments underlying each data point
- Figures may not include error bars for experiments with n<3; scatter plots showing individual data points should be used instead.

3) Revised manuscript text (including main tables, and figure legends for main and EV figures) has to be submitted as editable text file (e.g., .docx format). We encourage highlighting of changes (e.g., via text color) for the referees' reference.

4) Each main and each Expanded View (EV) figure should be uploaded as individual production-quality files (preferably in .eps, .tif, .jpg formats). For suggestions on figure preparation/layout, please refer to our Figure Preparation Guidelines:

http://bit.ly/EMBOPressFigurePreparationGuideline

5) Point-by-point response letters should include the original referee comments in full together with your detailed responses to them (and to specific editor requests if applicable), and also be uploaded as editable (e.g., .docx) text files.

6) Please complete our Author Checklist, and make sure that information entered into the checklist is also reflected in the manuscript; the checklist will be available to readers as part of the Review Process File. A download link is found at the top of our Guide to Authors: embopress.org/page/journal/14602075/authorguide

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In the interest of ensuring the conceptual advance provided by the work, we recommend submitting a revision within 3 months (10th Oct 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

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Referee #1:

How densely packaged regions of the genome are licensed for replication is poorly understood. Here, Sahu and colleagues investigate the interactions between ORC-associated protein (ORCA), ORC and nucleosomes containing trimethylated K20 H4, which regulates timely replication of heterochromatic domains. To do so, they determined the crystal structure of ORCA bound to an N-terminal Orc2 segment, explaining the stabilising effect of Orc2 on ORCA. They also determined the cryo-EM structure of a mononucleosome containing H4K20me3 bound to ORCA and Orc2N. The structure allows describing specific interactions between an aromatic cage contained in the ORCA WD40 domain and the methylated histone tail, and a basic patch in ORCA that engages nucleosomal DNA. Chromatin pelleting and phase separation assays show that ORCA is selectively recruited to chromatin containing methylated histones and that the binding of the WD40 domain reduces chromatin compaction. Other ORCA domains, particularly a linker containing basic residues, appear to counteract this effect, possibly by bridging adjacent nucleosomes. The structural biology and biochemistry is performed to very high standards and the work significantly increases our understanding of how ORC can be directed to heterochromatic regions via ORCA recruitment to H4K20me3-chromatin. It also shows how ORCA itself functions to unpackage chromatin locally, clearing the way for MCM double hexamer loading that licenses replication origin. This provides important conceptual advance that merits to be published in EMBO journal, after addressing a few minor issues.

1. Lines 151-155 and Figure 2e-f, S6d: The peptide array show binding of the isolated WD40 domain to specific histone sequences. It would be informative to add letters and numbers to the peptide array grid in figure 2e, so that it can be interpreted according to the MODified Histone Peptide Array manual. Also, the authors comment on the observation that methylation of the arginine that precedes the trimethylated lysine in H4K20me3-, H3K9me3- and H3K27me3-containing peptides, impairs ORCA binding. The authors might also want to comment on the effect that phosphorylation of the downstream serine and threonine have.

2. Line 194. "Therefore, we reconstituted chromatin arrays bearing 12 nucleosomes (referred to as chromatin hereafter)," It would be good to clarify here how the DNA sequence was chosen to support reconstitution of a 12-nucleosome array. The information is contained in the methods section only. I suggest the main text should explain that the chromatin array was assembled using "12 repeats of Widom 601 nucleosome positioning sequences separated by 30 base pairs".
3. Line 220 "A classic assay used extensively" seems redundant. An assay used extensively or a classic assay would be better.
4. Line 308: "used performed" should read "used".
5. Page 12 and elsewhere. It seems that, to avoid repetition, the WD40 domain is referred to as WD40 fold. When the domain is truncated, the authors refer to "absence of WD40 fold". I find this confusing - it is unclear whether the authors actually mean truncation of a domain or whether the protein sequence is there but it does not fold. I suggest the authors could refer to the absence of a WD40 module instead.
6. Page 15 and elsewhere. Unlike yeast proteins, human proteins should be referred to as MCM2-7 and not Mcm2-7.
7. Page 20 a reference is missing when stating that BAH domain of ORC1 is dispensable in Drosophila.
8. Figure S4a, scale bar should be added.
9. Figure S4c one of the 3D classes obtained from focused classification showed two ORCA bound, one per face of the nucleosome. I am surprised that no symmetry expansion approach is mentioned to try to boost the number of ORCA bound particles. Have the authors not bothered with trying that given that the resolution of the structure is already an impressive 2.9A or did the authors try and symmetry expansion did not help? Or was symmetry imposition avoided in the first place given that the protein, but not the DNA sequence are symmetrical in a nucleosome? This is my curiosity, and not a critique of the work.

Referee #2:

This submission reports structural studies characterizing the interactions between nucleosome, the ORC2 subunit of the Origin Recognition Complex, and the chromatin-associated protein ORCA/LRWD1. Using crystal and cryo-EM tools, the studies identify the ORCA's WD40 domain as a H3K20Me3 dependent nucleosome-binding moiety that prevents chromatin condensation and facilitates the recruitment of ORC in vitro on heterochromatic nucleosomes. These studies address the important question of how chromosome duplication is orchestrated on chromatin, facilitating the transfer of both genetic and epigenetic information during cell division, and specifically tackle the mechanism by which replication origins are determined and activated in a heterochromatin context. Although the involvement of ORCA in replication initiation at heterochromatic origins was reported previously, the current submission provides a structural and mechanistic basis to the association of ORCA, ORC and modified nucleosomes and furthers our understanding of molecular interactions that regulate replication initiation.

The paper is nicely written, the experimental design is clearly described, and the conclusions are discussed in the context of recent observations in the field. The proposed mechanistic implications, including the important prediction that ORCA binding might locally alter chromatin architecture, could be further tested in cells by mapping chromatin interactions and chromatin compaction on chromatin. However, the structural in vitro studies reported in the paper in its current form consist of an important contribution to the scientific literature and can further advance the field.

Minor suggestions:
Figure 2b: there seems to be a faint signal in the binding assay with H4K20Me3 (upper left panel) for the 0.25-1 micromolar concentrations but not lower or higher. Is this reproducible?
Legend to figure 4g: check the sentence in parentheses, "chosen to do be in"...?

Referee #3:

This study explores the biochemical and structural bases for the role of ORCA in the recruitment of the ORC complex to chromatin with the characteristics of compacted chromatin. Several previously published articles have highlighted the involvement of the H4K20me3 and K3K9me3 histone marks in ORCA recruitment and the assembly of origins that fire at the end of the S-phase. However, this new work considerably reinforces these observations by providing clear data on the recognition mechanisms between ORCA and Orc2 and between ORCA and condensed chromatin carrying the H4K20me3 histone mark. In addition, this work brings the new observation that binding of ORCA's WD40 domain to nucleosomes prevents chromatin array compaction in a manner that relies on tri-methylation of histone H4 at lysine 20 while adjacent ORCA regions stabilize the protein on chromatin. This last result is important for the field since it provides a clue on how ORCA creates a favorable environment for Mcm2-7 loading and probably also for the firing of pre-RC complexes in late S-phase. This observation reinforces those showing that, in general, active replication origins are found in more open chromatin contexts.
As I am not at all specialized in structural analysis, it is difficult for me to assess the quality of the structural analysis of the ORCA-Orc2 and the ORCA-Orc2-H4K20me3 nucleosome complexes. However, the data are easy to follow as presented. The interesting observation that the ORCA-binding peptide sequence in Orc2 is conserved with the occurrence of an ORCA ortholog suggests that their interaction plays an important role.
The use of chromatin arrays bearing 12 nucleosomes allows the author to robustly test the impact of ORCA and its mutants on chromatin bearing or not the H4K20me3 modification. Results are very convincing and show that multiple domains in ORCA

cooperate to recruit and stabilize ORCA on polynucleosomes in a histone modification-specific manner.
Then the authors used a well-established method to test the impact of ORCA recruitment on chromatin compaction. The impact of the full-length ORCA on chromatin pelleting although faint is reproducible and specific to the H4K20me3-modified nucleosome array. The effect is stronger with the ORCA WD40 domain and this different behavior is further sustained by experiments described later in the paper. Again, I am not familiar with this type of experiment and only experts can discuss whether the effect observed might have some relevance in vivo but this result has important implications since it suggests that the interaction of the ORCA WD40 domain with H4K20me3 modified histone tails inhibits chromatin compaction.
To further validate this observation, the authors used an assay based on the formation of chromatin droplets in the presence or not of the ORCA WD40 domain. They observed an inhibition of liquid-liquid phase separation (LLPS) when the WD40 domain of ORCA is incubated with chromatin carrying H4K20me3. The different behavior of the full-length ORCA, ie no effect on LLPS, suggests that the linker region contributes also to ORCA properties. Indeed, deletions of conserved residues found in the linker region affect the behavior of the full-length ORCA with both the chromatin pelleting and the LLPS assays.
The authors also use confocal microscopy to explore the distribution of mCherry-tagged ORCA and then GFP-tagged ORC within droplets. They observe that ORCA is essential and sufficient to specifically target ORC to condensed chromatin carrying H4K20me3 in vitro.
In conclusion, this paper explores the important question of understanding how the ORCA protein assists the replication machinery to ensure the initiation of replication in regions of condensed chromatin that are replicated at the end of S phase. To a non-expert, the biochemical and structural approaches used seem to be of very high quality. They provide clear and novel answers on the function of ORCA.

Response to reviewers:

We thank the referees for their time and careful review of our manuscript. We are grateful for their positive sentiments and enthusiasm for our work.

In the enclosed revision, we have addressed all concerns and suggestions of the referees. A point-by-point response to the issues raised is detailed in blue below and related changes are highlighted in the revised manuscript text.

Referee #1:

How densely packaged regions of the genome are licensed for replication is poorly understood. Here, Sahu and colleagues investigate the interactions between ORC-associated protein (ORCA), ORC and nucleosomes containing trimethylated K20 H4, which regulates timely replication of heterochromatic domains. To do so, they determined the crystal structure of ORCA bound to an N-terminal Orc2 segment, explaining the stabilising effect of Orc2 on ORCA. They also determined the cryo-EM structure of a mononucleosome containing H4K20me3 bound to ORCA and Orc2N. The structure allows describing specific interactions between an aromatic cage contained in the ORCA WD40 domain and the methylated histone tail, and a basic patch in ORCA that engages nucleosomal DNA. Chromatin pelleting and phase separation assays show that ORCA is selectively recruited to chromatin containing methylated histones and that the binding of the WD40 domain reduces chromatin compaction. Other ORCA domains, particularly a linker containing basic residues, appear to counteract this effect, possibly by bridging adjacent nucleosomes. The structural biology and biochemistry is performed to very high standards and the work significantly increases our understanding of how ORC can be directed to heterochromatic regions via ORCA recruitment to H4K20me3-chromatin. It also shows how ORCA itself functions to unpackage chromatin locally, clearing the way for MCM double hexamer loading that licenses replication origin. This provides important conceptual advance that merits to be published in EMBO journal, after addressing a few minor issues.

We thank the referee for the positive assessment of our work and for supporting publication in EMBO Journal.

1. Lines 151-155 and Figure 2e-f, S6d: The peptide array show binding of the isolated WD40 domain to specific histone sequences. It would be informative to add letters and numbers to the peptide array grid in figure 2e, so that it can be interpreted according to the MODified Histone Peptide Array manual.

We thank the referee for this suggestion and have added the labeling to all array figures (Fig 2E and Fig EV2D).

Also, the authors comment on the observation that methylation of the arginine that precedes the trimethylated lysine in H4K20me3-, H3K9me3- and H3K27me3-containing peptides, impairs ORCA binding. The authors might also want to comment on the effect that phosphorylation of the downstream serine and threonine have.

We have updated the sentence in the revised manuscript to comment on the negative impact of serine and threonine phosphorylation on ORCA binding. The sentence now reads

“Interestingly, ORCA binding to histone peptides with repressive lysine trimethylations is inhibited when the arginine N-terminal to the trimethylated lysine is itself methylated, or when the downstream serine and threonine in H3 are phosphorylated (**Fig 2F**).”

2. Line 194. "Therefore, we reconstituted chromatin arrays bearing 12 nucleosomes (referred to as chromatin hereafter)," It would be good to clarify here how the DNA sequence was chosen to support reconstitution of a 12-nucleosome array. The information is contained in the methods section only. I suggest the main text should explain that the chromatin array was assembled using "12 repeats of Widom 601 nucleosome positioning sequences separated by 30 base pairs".

We have updated the main manuscript text on page 10 to include this information.

3. Line 220 "A classic assay used extensively" seems redundant. An assay used extensively or a classic assay would be better.

We agree with the referee and have removed the word “classic” from the sentence.

4. Line 308: "used performed" should read "used".

Line 308 (page 15 in revised manuscript) does not contain the word “performed” but “preformed”. Since the use of preformed chromatin condensates is important for the experiments described in this paragraph, we have left the sentence unchanged.

5. Page 12 and elsewhere. It seems that, to avoid repetition, the WD40 domain is referred to as WD40 fold. When the domain is truncated, the authors refer to "absence of WD40 fold". I find this confusing - it is unclear whether the authors actually mean truncation of a domain or whether the protein sequence is there but it does not fold. I suggest the authors could refer to the absence of a WD40 module instead.

We thank the referee for raising this issue and have replaced “fold” by “module” on pages 13, 16, and the figure legend of Fig 3.

6. Page 15 and elsewhere. Unlike yeast proteins, human proteins should be referred to as MCM2-7 and not Mcm2-7.

We have modified the capitalization to MCM2-7 throughout the manuscript.

7. Page 20 a reference is missing when stating that BAH domain of ORC1 is dispensable in Drosophila.

We apologize for this oversight and have added the citation (now on page 21 of the revised manuscript).

8. Figure S4a, scale bar should be added.

We have added a scale bar to Figure S4a (now Appendix Figure S3A).

9. Figure S4c one of the 3D classes obtained from focused classification showed two ORCA bound, one per face of the nucleosome. I am surprised that no symmetry expansion approach is mentioned to try to boost the number of ORCA bound particles. Have the authors not bothered with trying that given that the resolution of the structure is already an impressive 2.9A or did the authors try and symmetry expansion did not help? Or was symmetry imposition avoided in the first place given that the protein, but not the DNA sequence are symmetrical in a nucleosome? This is my curiosity, and not a critique of the work.

We thank the referee for this comment. We had indeed tried to improve the resolution by symmetry expansion but found that the ORCA map region was not improved. Since the DNA in the nucleosome used is not symmetric, we chose to use the non-symmetry-expanded maps for model building and structure interpretation.

Referee #2:

This submission reports structural studies characterizing the interactions between nucleosome, the ORC2 subunit of the Origin Recognition Complex, and the chromatin-associated protein ORCA/LRWD1. Using crystal and cryo-EM tools, the studies identify the ORCA's WD40 domain as a H3K20Me3 dependent nucleosome-binding moiety that prevents chromatin condensation and facilitates the recruitment of ORC in vitro on heterochromatic nucleosomes. These studies address the important question of how chromosome duplication is orchestrated on chromatin, facilitating the transfer of both genetic and epigenetic information during cell division, and specifically tackle the mechanism by which replication origins are determined and activated in a heterochromatin context. Although the involvement of ORCA in replication initiation at heterochromatic origins was reported previously, the current submission provides a structural and mechanistic basis to the association of ORCA, ORC and modified nucleosomes and furthers our understanding of molecular interactions that regulate replication initiation.

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We thank the referee for appreciating our structural and *in vitro* biochemical findings.

Minor suggestions:

Figure 2b: there seems to be a faint signal in the binding assay with H4K20Me3 (upper left panel) for the 0.25-1 micromolar concentrations but not lower or higher. Is this reproducible?

We are not entirely sure which figure the referee is referring to since Figure 2b does not show a binding assay but the cryo-EM structure. If the referee means Figure 4b instead, then yes, the reduced pelleting efficiency in the presence of 0.25-1 μM full-length ORCA is reproducible. The corresponding quantification for the 0.5 μM concentration is shown as inset in Figure 4E.

Legend to figure 4g: check the sentence in parentheses, "chosen to do be in"...?

We thank the referee for spotting this mistake and have corrected it.

Referee #3:

This study explores the biochemical and structural bases for the role of ORCA in the recruitment of the ORC complex to chromatin with the characteristics of compacted chromatin. Several previously published articles have highlighted the involvement of the H4K20me3 and K3K9me3 histone marks in ORCA recruitment and the assembly of origins that fire at the end of the S-phase. However, this new work considerably reinforces these observations by providing clear data on the recognition mechanisms between ORCA and Orc2 and between ORCA and condensed chromatin carrying the H4K20me3 histone mark. In addition, this work brings the new observation that binding of ORCA's WD40 domain to nucleosomes prevents chromatin array compaction in a manner that relies on tri-methylation of histone H4 at lysine 20 while adjacent ORCA regions stabilize the protein on chromatin. This last result is important for the field since it provides a clue on how ORCA creates a favorable environment for Mcm2-7 loading and probably also for the firing of pre-RC complexes in late S-phase. This observation reinforces those showing that, in general, active replication origins are found in more open chromatin contexts.

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We thank the referee for complementing on the high quality of our work and are delighted that the reviewer agrees that the results provide new insights into the function of ORCA in replication initiation.

Dr. Franziska Bleichert
Yale University
Molecular Biophysics and Biochemistry
260 Whitney Avenue
YSB 345
New Haven, CT 06520

24th Jul 2023

Re: EMBOJ-2023-114654R
A Dual Role for the Chromatin Reader ORCA/LRWD1 in Targeting the Origin Recognition Complex to Chromatin

Dear Dr. Bleichert,

Thank you for submitting your revised manuscript to The EMBO Journal. Having now assessed your responses to the original reports, I am happy to say that we are ready to accept the study for publication, following incorporation of the following editorial points:

- Pre-acceptance checks by our data editors have raised several queries with the data descriptors in the figure legends, which you will find as comments in the attached edited/commented Word document with activated "Track changes" option. I would appreciate if you incorporated the requested final text modifications and answered the Figure legend queries directly in this version (and modified figures where necessary), uploading the edited main text document upon resubmission with changes/additions still highlighted via the "Track changes" option, to facilitate our final checking.

- Regarding the 3 included tables: If they should published as regular tables in the main text, they should be included in the main text file and be formatted as editable text-only (no colors or grey-scale possible). Alternatively, you could upload them as 3 individual Expanded View tables (nomenclature in the files and when referencing them in the text: "Table EV1/2/3"), or add them to the Appendix PDF (where they would need to be called out also in the Table of Contents, as "Appendix Table S1/2/3"). In both of these cases, the formatting/shading etc. could be retained.

- Please correct the reference list, making sure that for references with multiple authors, the first up to 10 authors should be listed, followed by 'et al.' after that (please refer to our Guide to Authors for additional information on EMBO J reference format).

- As we are switching from a free-text author contribution statement towards a more formal statement based on Contributor Role Taxonomy (CRediT) terms, please remove the present Author Contribution section and instead specify each author's contribution(s) directly in the Author Information page of our submission system during upload of the final manuscript. See https://casrai.org/credit/ for more information.

- Please make sure to include all relevant funding information both in the manuscript and in our submission system. This info appears to be missing in the system: Endowed Rudolph Anderson Fellowship from Yale University, Swiss National Science Foundation Postdoc. Mobility fellowship, Leslie H. Warner Postdoctoral Fellowship, Yale Cancer Center

- Please provide suggestions for a short 'blurb' text prefacing and summing up the conceptual aspect of the study in two sentences (max. 250 characters), followed by 3-5 one-sentence 'bullet points' with brief factual statements of key results of the paper; they will form the basis of an editor-written 'Synopsis' accompanying the online version of the article. Please also upload a synopsis image, which can be used as a "visual title" for the synopsis section of your paper - maybe simply based on Figure 7? The image should be in PNG or JPG format, and please make sure that it remains in the modest dimensions of (exactly) 550 pixels wide and 300-600 pixels high.

- Finally, please ensure that all deposited datasets listed in the "Data Availability" section are being publicly released at this point - in particular the structural data.

I am therefore returning the manuscript to you for a final round of minor revision, to allow you to make these adjustments and upload all modified files. Once we will have received them, we should be ready to swiftly proceed with formal acceptance and production of the manuscript.

Yours sincerely,

Hartmut Vodermaier

Hartmut Vodermaier, PhD
Senior Editor, The EMBO Journal
h.vodermaier@embojournal.org

*** PLEASE NOTE: All revised manuscript are subject to initial checks for completeness and adherence to our formatting guidelines. Revisions may be returned to the authors and delayed in their editorial re-evaluation if they fail to comply to the following requirements (see also our Guide to Authors for further information):

1) Every manuscript requires a Data Availability section (even if only stating that no deposited datasets are included). Primary datasets or computer code produced in the current study have to be deposited in appropriate public repositories prior to resubmission, and reviewer access details provided in case that public access is not yet allowed. Further information: embopress.org/page/journal/14602075/authorguide#dataavailability

2) Each figure legend must specify
- size of the scale bars that are mandatory for all micrograph panels
- the statistical test used to generate error bars and P-values
- the type error bars (e.g., S.E.M., S.D.)
- the number (n) and nature (biological or technical replicate) of independent experiments underlying each data point
- Figures may not include error bars for experiments with n<3; scatter plots showing individual data points should be used instead.

3) Revised manuscript text (including main tables, and figure legends for main and EV figures) has to be submitted as editable text file (e.g., .docx format). We encourage highlighting of changes (e.g., via text color) for the referees' reference.

4) Each main and each Expanded View (EV) figure should be uploaded as individual production-quality files (preferably in .eps, .tif, .jpg formats). For suggestions on figure preparation/layout, please refer to our Figure Preparation Guidelines: http://bit.ly/EMBOPressFigurePreparationGuideline

5) Point-by-point response letters should include the original referee comments in full together with your detailed responses to them (and to specific editor requests if applicable), and also be uploaded as editable (e.g., .docx) text files.

6) Please complete our Author Checklist, and make sure that information entered into the checklist is also reflected in the manuscript; the checklist will be available to readers as part of the Review Process File. A download link is found at the top of our Guide to Authors: embopress.org/page/journal/14602075/authorguide

7) All authors listed as (co-)corresponding need to deposit, in their respective author profiles in our submission system, a unique ORCiD identifier linked to their name. Please see our Guide to Authors for detailed instructions.

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9) Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be clearly noted in the figure legend and/or the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure. Finally, we generally encourage uploading of numerical as well as gel/blot image source data; for details see: embopress.org/page/journal/14602075/authorguide#sourcedata

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In the interest of ensuring the conceptual advance provided by the work, we recommend submitting a revision within 3 months (22nd Oct 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

Link Not Available

The authors have addressed all minor editorial requests.

Dr. Franziska Bleichert
Yale University
Molecular Biophysics and Biochemistry
260 Whitney Avenue
YSB 345
New Haven, CT 06520

26th Jul 2023

Re: EMBOJ-2023-114654R1
A Dual Role for the Chromatin Reader ORCA/LRWD1 in Targeting the Origin Recognition Complex to Chromatin

Dear Dr. Bleichert,

Thank you for submitting your final revised manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.

Your article will be processed for publication in The EMBO Journal by EMBO Press and Wiley, who will contact you with further information regarding production/publication procedures and license requirements. You will also be provided with page proofs after copy-editing and typesetting of main manuscript and expanded view figure files.

Should you be planning a Press Release on your article, please get in contact with embojournal@wiley.com as early as possible, in order to coordinate publication and release dates.

Thank you again for this contribution to The EMBO Journal and congratulations on a successful publication! Please consider us again in the future for your most exciting work.

Yours sincerely,

Hartmut Vodermaier

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EMBO Press Author Checklist

Corresponding Author Name: Franziska Bleichert
Journal Submitted to: EMBO Journal
Manuscript Number: EMBOJ-2023-114654

USEFUL LINKS FOR COMPLETING THIS FORM

The EMBO Journal - Author Guidelines
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Reporting Checklist for Life Science Articles (updated January

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your manuscript.

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for 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.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

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.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Yes	Data Availability Statement

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Materials and Methods

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Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Not Applicable	

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Microbes: provide species and strain, unique accession number if available, and source.	Yes	Materials and Methods

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If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	Acknowledgements

Design

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Include a statement about **sample size** estimate even if no statistical methods were used.	Not Applicable	
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Not Applicable	
Include a statement about **blinding** even if no blinding was done.	Not Applicable	
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Yes	Cryo-EM particle exclusion/inclusion was done according to standards in the field and is included in the Supplemental Figures and Materials and Methods.
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Yes	Figure legends

Sample definition and in-laboratory replication	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	Yes	Number of repeats are included in the figures or Materials and Methods section.
In the figure legends: define whether data describe **technical or biological replicates**.	Not Applicable	

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Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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Studies involving **human participants**: 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.	Not Applicable	
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental **animals**: State details of **authority granting ethics approva**l (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
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The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

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For **phase II and III randomized controlled trials**, please refer to the **CONSORT** flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Yes	Data Availability Statement
If publicly available data were reused, provide the respective **data citations in the reference list**.	Yes	Figure legends, Materials and Methods, and References