

Peer Review Information

Journal: Nature Structural & Molecular Biology

Manuscript Title: Guiding the HBO1 complex function through the JADE subunit

Corresponding author name(s): Tatiana G. Kutateladze, Jacques Côté, Akihiko Yokoyama

Reviewer Comments & Decisions:

Decision Letter, initial version:
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Message: 22nd Jun 2023

Dear Professor Kutateladze,

Thank you again for submitting your manuscript "Guiding the HBO1 complex function through the JADE subunit". I apologize for the delay in responding, which resulted from the difficulty in obtaining suitable referee reports. Nevertheless, we now have comments (below) from the 3 reviewers who evaluated your paper. In light of those reports, we remain interested in your study and would like to see your response to the comments of the referees, in the form of a revised manuscript.

You will see that while all 3 reviewers appreciated the work, Reviewer #1 requests strengthening the mechanistic model by quantifying the relative H3 and H4 acetylation activity, expanding on the functional meaning of the interaction of JADE1 with H3 on H4 acetylation, and to address this activity in the context of di-nucleosomes and chromatin in cells. Additionally, Reviewer #2 requests placing the results more appropriately in the context of the literature and ensuring data availability and accessibility, and Reviewer #3 requests expanding on the characterization of the PZP domain -histone interaction. Please be sure to address/respond to all concerns of the referees in full in a point-by-point response and highlight all changes in the revised manuscript text file. Please ensure that all raw data has been made accessible to the Reviewers in the next round and include accession codes and reviewer tokens in a Data Availability Statement in the revised manuscript.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

We appreciate the requested revisions are extensive. We thus expect to see your revised manuscript within 6 months. If you cannot send it within this time, please let us know. We will be happy to consider your revision as long as nothing similar has been accepted for

publication at NSMB or published elsewhere. Should your manuscript be substantially delayed without notifying us in advance and your article is eventually published, the received date would be that of the revised, not the original, version.

As you already know, we put great emphasis on ensuring that the methods and statistics reported in our papers are correct and accurate. As such, if there are any changes that should be reported, please submit an updated version of the Reporting Summary along with your revision.

Reporting Summary:

<https://www.nature.com/documents/nr-reporting-summary.pdf>

Please note that the form is a dynamic 'smart pdf' and must therefore be downloaded and completed in Adobe Reader.

When submitting the revised version of your manuscript, please pay close attention to our [href="https://www.nature.com/nature-portfolio/editorial-policies/image-integrity">Digital Image Integrity Guidelines.](https://www.nature.com/nature-portfolio/editorial-policies/image-integrity) and to the following points below:

- that unprocessed scans are clearly labelled and match the gels and western blots presented in figures.
- that control panels for gels and western blots are appropriately described as loading on sample processing controls
- all images in the paper are checked for duplication of panels and for splicing of gel lanes.

Finally, please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

If there are additional or modified structures presented in the final revision, please submit the corresponding PDB validation reports.

Please note that all key data shown in the main figures as cropped gels or blots should be presented in uncropped form, with molecular weight markers. These data can be aggregated into a single supplementary figure. While these data can be displayed in a relatively informal style, they must refer back to the relevant figures. These data should be submitted with the last revision, prior to acceptance, but you may want to start putting it together at this point.

SOURCE DATA: we request that authors provide, in tabular form, the data underlying the graphical representations used in figures. This is to further increase transparency in data reporting, as detailed in this editorial (<http://www.nature.com/nsmb/journal/v22/n10/full/nsmb.3110.html>). Spreadsheets can be submitted in excel format. Only one (1) file per figure is permitted; thus, for multi-paneled figures, the source data for each panel should be clearly labeled in the Excel file; alternately the data can be provided as multiple, clearly labeled sheets in an Excel file. When submitting files, the title field should indicate which figure the source data pertains to. We encourage our authors to provide source data at the revision stage, so that they are part of the

peer-review process.

While we encourage the use of color in preparing figures, please note that this will incur a charge to partially defray the cost of printing. Information about color charges can be found at <http://www.nature.com/nsmb/authors/submit/index.html#costs>

We require deposition of coordinates (and, in the case of crystal structures, structure factors) into the Protein Data Bank with the designation of immediate release upon publication (HPUB). Electron microscopy-derived density maps and coordinate data must be deposited in EMDB and released upon publication. Deposition and immediate release of NMR chemical shift assignments are highly encouraged. Deposition of deep sequencing and microarray data is mandatory, and the datasets must be released prior to or upon publication. To avoid delays in publication, dataset accession numbers must be supplied with the final accepted manuscript and appropriate release dates must be indicated at the galley proof stage. Please find the complete NRG policies on data availability at <http://www.nature.com/authors/policies/availability.html>.

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Please use the link below to submit your revised manuscript and related files:

[Redacted]

Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,
Sara

Sara Osman, Ph.D.
Associate Editor
Nature Structural & Molecular Biology

Referee expertise:

Referee #1: Structural biology, Epigenetics

Referee #2: Epigenomics, Cancer

Referee #3: NMR, Cancer

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

In this manuscript, Gaurav et al. reported their structural and functional analyses of JADE subunit of the HBO1 complex in gene transcription in chromatin. Specifically, they provided comprehensive characterization of the structural and molecular basis of the PZP domain of JADE interactions with histone H3 in both sequence and H3K4 methylation dependent manner, as well as the nucleosomal DNA. They further demonstrated the importance of the PZP domain recognition of the unmethylated H3K4 for facilitating the enzymatic activity of the HBO1 complex towards histone H4 lysine acetylation, as well as how H3K4 methylation compromises the HBO1's histone acetylation activity for H4. Additionally, the authors provided cellular data illustrating how the PZP domain functions of the NUP98-JADE2 fusion protein contribute to leukemogenesis. Overall, this is an interesting study, as it provides new insights into the regulatory mechanism that governs histone H4 acetylation by the HBO1 complex through its JADE subunit interactions with unmodified histone H3K4. These findings have important implications in better understanding of gene transcriptional activation in chromatin. There are a few concerns regarding their mechanistic model that the authors should address, which as described as follows.

1. The mechanistic models depicted in Figure 2j, 2k appear to be supported by the data presented in Figure 2d. However, given that the western blot data are not quantified, it is not clear how significant the H3 acetylation activity is as compared to the activity on H4 acetylation. Therefore, it is recommended that the authors provide quantifications for the histone H3 and H4 acetylation activities under the various conditions and adjust their conclusions accordingly.
2. While H3K4 methylation marked reduces JADE1 binding to the N-terminal region of H3, JADE1 can still bind histone H3 through interacting with H3 residues 15-34 (Figure 4), as well as DNA in nucleosome (Figure 5). Therefore, the authors need to explain whether or how the latter two molecular interactions of JADE1 have any functional significance in the HBO1 complex's histone H4 lysine acetylation activity (Figure 2).
3. It will also be important for the authors to address the role of the HBO1 complex's lysine acetylation activity of histone H4 in the context of di-nucleosome and in chromatin cells. Their mechanistic model presented in Figure 2 was derived only from their data generated using mono-nucleosomes.

Reviewer #2:

Remarks to the Author:

The overall goal of this study was to determine how the JADE components of the HBO1 KAT complex contribute to target specificity of the complex. In an overexpression experiment, the authors found that JADE1-3 IP's with MLL1. ChIP-seq showed some co-localization of these complexes on chromatin. Purified JADE/HBO1 complexes with or

without ING4/5 and MEAF6 showed differential acetylation activities on H3K4 methylated nucleosomes. More specifically, H3K4me3 drives acetylation towards H3 and completely blocks KAT activity of the JADEs/HBO1 complex. Structural analysis indicates that the JADE PZP domain can engage with the unmodified H3 tail region at two sites. The authors also identified a non-specific DNA binding activity of two positively charged patches on the JADE protein. Using a combination of IF, fractionation and ChIP, the authors also found that ING5/HBO1 caused increased localisation of JADE to the nucleus and the JADE3 PZP domain was essential for stable chromatin binding. Finally, mutations of the PZP domain prevented NUP98-JADE2 fusions from transforming bone marrow cells.

Overall, this is an interesting paper analysing the crosstalk of KMT and KAT complexes, and provides some interesting new insights for the field. I just have a few questions and concerns:

1. The first statement in the introduction "Histone acetylation promotes chromatin decondensation..." should be supported with specific paper references, as the role of histone acetylation in the cell is still controversial.
2. Figure 1 and S1 show an association of MLL1 with overexpressed JADE components. Does JADE/HBO1 associate with other factors from the MLL1 complex (e.g. WRAD, MENIN, HCFC1)? What about other KMT complexes such as SET1?
3. Can the authors provide evidence that endogenous levels of JADE proteins interact with endogenous MLL complexes, or is this an effect driven by JADE/HBO1 overexpression?
4. Can the authors use their genomic data (Figure 1) to better quantify the co-localization of MLL1 and the HBO1 complex, with scatter plots or with a signal correlation matrix?
5. For the ChIP-seq experiments in Figure 7, can the authors verify that there are equal levels of JADE3 and JADE3dPZP in the nucleus (e.g. western blot)? It looks like JADE3dPZP is less stable and expressed at lower levels, which could be contributing to the reduced ChIP-seq signal.
6. Why is JADE2 transforming but JADE1 and 3 are not, considering how similar in sequence these proteins are?
7. I tried to access the UCSC session to examine the ChIP-seq but for some reason I received this error message: "Could not find session Guiding the HBO1 compIex function through the JADE subunit for user akkanai@edu.k.u-tokyo.ac.jp." A proper session link should be provided so the reviewers can view the raw ChIP-seq data for themselves. It would be good to provide the link in the paper itself, as it was difficult to extract from the reporting summary.

Reviewer #3:

Remarks to the Author:

I thoroughly enjoyed reading this manuscript and believe it holds the potential to make a significant impact on multiple fields if appropriately revised. My comments are as follows:

- 1) The manuscript provides an insightful analysis of the PZP domain of JADE1, comprising two PHD zinc fingers (PHD1 and PHD2) that specifically bind to H3 1-34. The crystal structure elucidates the interaction between PHD1 and H3 1-12 with clarity. To further enrich the understanding of this complex, can the authors elaborate on the functional role of PHD2 and hopefully examine whether PHD2 subsequently binds to the same H3 15-34. Additionally, given the presence of two copies of H3 in the nucleosomal context, it would be necessary to find out whether the PZP domain binds to both H3 molecules

symmetrically (in a 2:2 ratio) or asymmetrically (in a 1:2 ratio). Quantitative data presenting the binding affinity will yield valuable insights into the interaction dynamics.

2) Within the region separating the two PHD zinc fingers responsible for histone binding, other zinc finger domains are known to interact with the linker DNA between nucleosomes. Exploring the ChIP-seq data (Figure 1D) could offer valuable clues in identifying the binding motif for these additional zinc fingers. Given the capability of all JADE1/2/3 proteins to recruit various components within the HBO1 complex, it is conceivable that they possess distinct binding motifs, facilitating their functionality at specific chromatin loci. Can the authors provide any clue of these potential differences?

3) The PHD finger domain serves as a crucial epigenetic reader, exhibiting the ability to bind various epigenetic marks. For instance, the ING2 PHD finger is known to bind H3K4me3, a heterochromatin marker. Conversely, the PHD1 domain explored in this study demonstrates a preference for hypomethylated H3K4, a euchromatin marker. Highlighting the distinctions between these two binding preferences would provide valuable insights into the functional diversity of PHD finger domains as epigenetic readers.

4) The manuscript would be substantially enhanced by the inclusion of a comprehensive model that illustrates the intricate interaction between the PZP domain and the nucleosome. Such a model would greatly aid in visualizing the structural and functional aspects of this complex interaction.

5) Have the authors assigned the resonances of Fig 2f that produced the K_d in Fig 2g? Similarly, Figs 4j and 4i? The chemical shift changes are so big whereas the higher concentrations induced tiny chemical shift changes due to slow exchange/tight binding. I wonder how the authors were able to trace the perturbed signals. Please provide more details.

Again, I think this is a very strong/interesting manuscript. With proper revision, the manuscript will significantly contribute to the advancement of knowledge in several fields especially in the chromatin field.

Author Rebuttal to Initial comments

We thank the Editor and Reviewers for the careful review of our manuscript and the insightful and very constructive comments, which were helpful in revising and strengthening this manuscript.

Reviewer 1, Comment 1: In this manuscript, Gaurav et al. reported their structural and functional analyses of JADE subunit of the HBO1 complex in gene transcription in chromatin. Specifically, they provided comprehensive characterization of the structural and molecular basis of the PZP domain of JADE interactions with histone H3 in both sequence and H3K4 methylation dependent manner, as well as the nucleosomal DNA. They further demonstrated the importance of the PZP domain recognition of the unmethylated H3K4 for facilitating the enzymatic activity of the HBO1 complex towards histone H4 lysine acetylation, as well as how H3K4 methylation compromises the HBO1's histone acetylation activity for H4. Additionally, the

authors provided cellular data illustrating how the PZP domain functions of the NUP98-JADE2 fusion protein contribute to leukemogenesis. Overall, this is an interesting study, as it provides new insights into the regulatory mechanism that governs histone H4 acetylation by the HBO1 complex through its JADE subunit interactions with unmodified histone H3K4. These findings have important implications in better understanding of gene transcriptional activation in chromatin. There are a few concerns regarding their mechanistic model that the authors should address, which as described as follows.

The mechanistic models depicted in Figure 2j, 2k appear to be supported by the data presented in Figure 2d. However, given that the western blot data are not quantified, it is not clear how significant the H3 acetylation activity is as compared to the activity on H4 acetylation. Therefore, it is recommended that the authors provide quantifications for the histone H3 and H4 acetylation activities under the various conditions and adjust their conclusions accordingly.

Author's response: we have quantified radioactive HAT assay data shown in Fig. 2d in Suppl. Fig. 3a, repeated the HAT assay with the unmodified and symmetrically H3K4me3-containing NCPs (Suppl. Fig. 3b), and carried out HAT assays using H3K4me3-rich yeast native chromatin as a substrate (Suppl. Fig. 3c). All HAT assays confirmed the shift toward H3 acetylation for the JADE1-ING4/5-MEAF6 containing HBO1 complex on hypermethylated (H3K4me3) substrates.

We also performed in vivo ChIP-qPCR assays in cells stably expressing JADEs and cells transiently expressing HBO1 complex components to confirm that the four-subunits HBO1 complex acetylates H3 and H4 at an active H3K4me3-enriched promoter, whereas HBO1-JADE1S is impaired in either histone acetylation. New data are shown in Suppl. Fig. 4, and new Fig. 5h has been added.

The following sentences have been added on pages 10-11: "The bivalent interaction of JADE1_{PZP} with a large portion of histone H3 and the linker DNA (Fig. 5h) can explain histone substrate selectivity of the HBO1 complex – binding to the almost entire H3 tail (unmethylated at K4) protects this tail from being acetylated, hence the HBO1 complex acetylates predominantly H4. Methylation of H3K4 disrupts binding of JADE1_{PZP}, removing such a protection and making H3 tail amenable to acetylation and binding of ING4/5."

Reviewer 1, Comment 2: While H3K4 methylation marked reduces JADE1 binding to the N-terminal region of H3, JADE1 can still bind histone H3 through interacting with H3 residues 15-34 (Figure 4), as well as DNA in nucleosome (Figure 5). Therefore, the authors need to explain whether or how the latter two molecular interactions of JADE1 have any functional significance in the HBO1 complex's histone H4 lysine acetylation activity (Figure 2).

Author's response: we have performed ChIP-qPCR assays using JADE1 Δ PHD2 to eliminate binding of JADE1 to the middle part of H3 and DNA. As shown in new Suppl. Fig. 4a the HAT activity of the HBO1 complex is substantially decreased.

The following sentences have been added on page 10: "...both H3- and DNA-binding functions are essential for the high affinity interaction with the nucleosome and for enzymatic activity of the JADE1-ING4/5-MEAF6-HBO1 complex. Eliminating binding to the middle part of H3 and DNA by deleting PHD2 in JADE1 (JADE1 Δ PHD2) substantially decreased the HAT activity of the complex in HeLa cells (Suppl. Fig. 4a)."

Reviewer 1, Comment 3: It will also be important for the authors to address the role of the HBO1 complex's lysine acetylation activity of histone H4 in the context of di-nucleosome and in chromatin cells. Their mechanistic model presented in Figure 2 was derived only from their data generated using mono-nucleosomes.

Author's response: to validate the HAT data on mononucleosomes, we used yeast native chromatin (oligonucleosomes) as a substrate in in vitro HAT assays and investigated acetylation in HeLa and HEK293T cells in vivo, please see our response to Comment 1. New data are shown in Suppl. Figs. 3b, 3c, 4a, and 4b.

Reviewer 2, Comment 1: The overall goal of this study was to determine how the JADE components of the HBO1 KAT complex contribute to target specificity of the complex. In an overexpression experiment, the authors found that JADE1-3 IP's with MLL1. ChIP-seq showed some co-localization of these complexes on chromatin. Purified JADE/HBO1 complexes with or without ING4/5 and MEAF6 showed differential acetylation activities on H3K4 methylated nucleosomes. More specifically, H3K4me3 drives acetylation towards H3 and completely blocks KAT activity of the JADEs/HBO1 complex. Structural analysis indicates that the JADE PZP domain can engage with the unmodified H3 tail region at two sites. The authors also identified a non-specific DNA binding activity of two positively charged patches on the JADE protein. Using a combination of IF, fractionation and ChIP, the authors also found that ING5/HBO1 caused increased localisation of JADE to the nucleus and the JADE3 PZP domain was essential for stable chromatin binding. Finally, mutations of the PZP domain prevented NUP98-JADE2 fusions from transforming bone marrow cells.

Overall, this is an interesting paper analysing the crosstalk of KMT and KAT complexes, and provides some interesting new insights for the field. I just have a few questions and concerns:

1. The first statement in the introduction "Histone acetylation promotes chromatin decondensation..." should be supported with specific paper references, as the role of histone acetylation in the cell is still controversial.

Author's response: the citations #1-4 have been added.

Reviewer 2, Comment 2: Figure 1 and S1 show an association of MLL1 with overexpressed JADE components. Does JADE/HBO1 associate with other factors from the MLL1 complex (e.g. WRAD, MENIN, HCFC1)? What about other KMT complexes such as SET1?

Author's response: new data shown in Suppl. Fig. 1b and bottom panel in Suppl. Fig. 1a confirm that endogenous and overexpressed JADE3 and HBO1 associate with MENIN.

Reviewer 2, Comment 3: Can the authors provide evidence that endogenous levels of JADE proteins interact with endogenous MLL complexes, or is this an effect driven by JADE/HBO1 overexpression?

Author's response: the association of endogenous JADE3, HBO1, MENIN and MLL1-C is shown in Suppl. Fig. 1b.

Reviewer 2, Comment 4: Can the authors use their genomic data (Figure 1) to better quantify the co-localization of MLL1 and the HBO1 complex, with scatter plots or with a signal correlation matrix?

Author's response: as suggested, the scatter plots of the ChIP signals at the individual genes have been added in Suppl. Fig. 1c.

Reviewer 2, Comment 5: For the ChIP-seq experiments in Figure 7, can the authors verify that there are equal levels of JADE3 and JADE3dPZP in the nucleus (e.g. western blot)? It looks like JADE3dPZP is less stable and expressed at lower levels, which could be contributing to the reduced ChIP-seq signal.

Author's response: new data shown in Suppl. Fig. 5a confirm roughly equal expression levels of JADE3 and JADE3ΔPZP (WCE).

Reviewer 2, Comment 6: Why is JADE2 transforming but JADE1 and 3 are not, considering how similar in sequence these proteins are?

Author's response: we agree with the reviewer, it is an interesting observation, and although we don't know why, we currently analyze other parts of JADE proteins that might be responsible for such a difference.

Reviewer 2, Comment 7: I tried to access the UCSC session to examine the ChIP-seq but for some reason I received this error message: "Could not find session Guiding the HBO1 complex function through the JADE subunit for user akkanai@edu.k.u-tokyo.ac.jp." A proper session link should be provided so the reviewers can view the raw ChIP-seq data for themselves. It would be good to provide the link in the paper itself, as it was difficult to extract from the reporting summary.

Author's response: we apologize, we are not sure why the access was denied to the reviewer. The corresponding authors, TK, AY and JC were able to access and see the raw data before submission of the manuscript.

Reviewer 3, Comment 1: I thoroughly enjoyed reading this manuscript and believe it holds the potential to make a significant impact on multiple fields if appropriately revised. My comments are as follows:

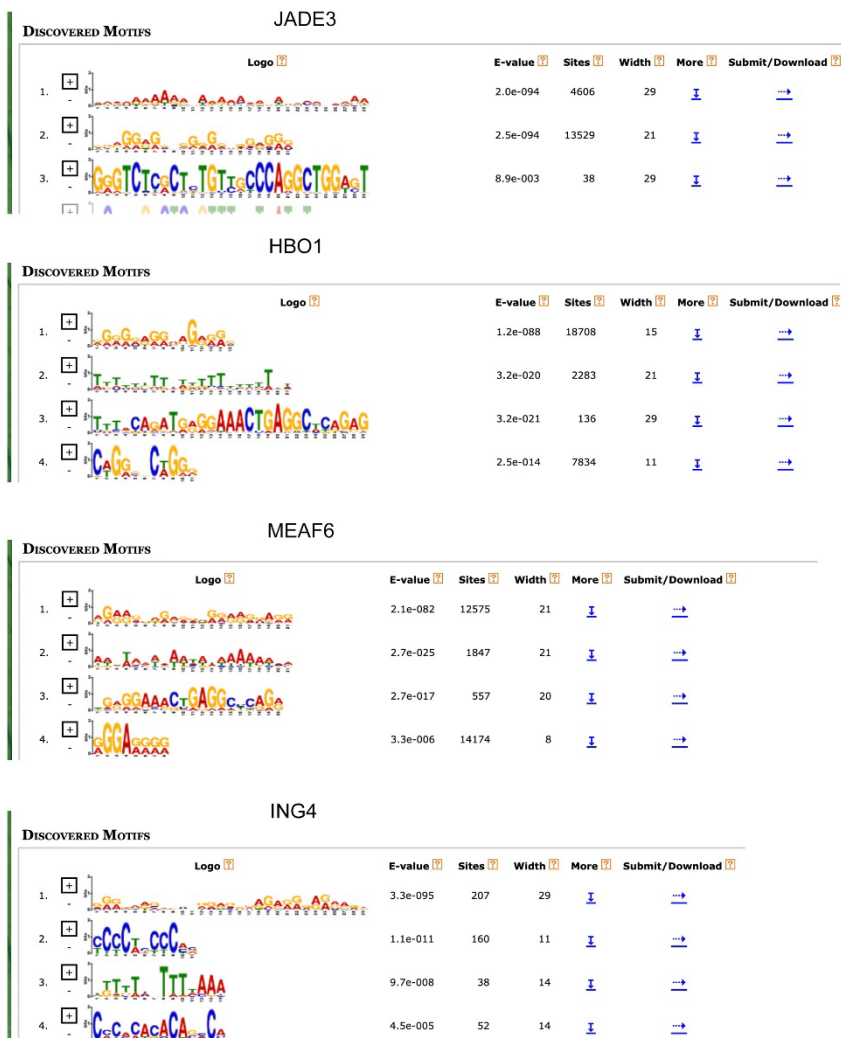
1) The manuscript provides an insightful analysis of the PZP domain of JADE1, comprising two PHD zinc fingers (PHD1 and PHD2) that specifically bind to H3 1-34. The crystal structure elucidates the interaction between PHD1 and H3 1-12 with clarity. To further enrich the understanding of this complex, can the authors elaborate on the functional role of PHD2 and hopefully examine whether PHD2 subsequently binds to the same H3 15-34. Additionally, given the presence of two copies of H3 in the nucleosomal context, it would be necessary to find out whether the PZP domain binds to both H3 molecules symmetrically (in a 2:2 ratio) or asymmetrically (in a 1:2 ratio). Quantitative data presenting the binding affinity will yield valuable insights into the interaction dynamics.

Author's response: because the binding groove for H3₁₅₋₃₄ is formed by all three domains (PHD1, PHD2 and Zn-kn), the interaction with H3₁₅₋₃₄ requires the intact PZP domain rather than individual PHD2 (E215K mutation that disrupts binding to H3₁₅₋₃₄ is a mutation in PHD1), and examining an individual PHD2 will not be informative. However, to determine the functional role of PHD2 *in vivo*, we carried out ChIP-qPCR assays in HeLa cells stably expressing JADE1 in which PHD2 is deleted. As shown in Suppl. Fig. 4a, the deletion of PHD2, i.e. elimination of binding of JADE1 to the middle part of H3 and DNA, led to a substantially decreased HAT activity of the complex.

The following sentences have been added on page 10: "...both H3- and DNA-binding functions are essential for the high affinity interaction with the nucleosome and for enzymatic activity of the JADE1-ING4/5-MEAF6-HBO1 complex. Eliminating binding to the middle part of H3 and DNA by deleting PHD2 in JADE1 (JADE1 Δ PHD2) substantially decreased the HAT activity of the complex in HeLa cells (Suppl. Fig. 4a)."

For the mode of this interaction: the fluorescence data shown in Fig. 4a, reveal a cooperative engagement of one PZP domain with two regions of one histone H3₁₋₃₁ tail, i.e., *in cis* binding, 1:1. We have added a reference (#21) to our previous study, where we characterized this *in cis* binding mechanism in detail.

Reviewer 3, Comment 2: Within the region separating the two PHD zinc fingers responsible for histone binding, other zinc finger domains are known to interact with the linker DNA between nucleosomes. Exploring the ChIP-seq data (Figure 1D) could offer valuable clues in identifying the binding motif for these additional zinc fingers. Given the capability of all JADE1/2/3 proteins to recruit various components within the HBO1 complex, it is conceivable that they possess distinct binding motifs, facilitating their functionality at specific chromatin loci. Can the authors provide any clue of these potential differences?



Author's response: as suggested, we analyzed our ChIP-seq data using the MEME program with the goal to identify potential binding motifs for the components of the HBO1 complex. The MEME program didn't identify any specific motifs (please see Figure on the left), therefore we do not discuss it further.

Reviewer 3, Comment 3: The PHD finger domain serves as a crucial epigenetic reader, exhibiting the ability to bind various epigenetic marks. For instance, the ING2 PHD finger is known to bind H3K4me3, a heterochromatin marker. Conversely, the PHD1 domain explored in this study demonstrates a preference for hypomethylated H3K4, a euchromatin marker. Highlighting the distinctions between these two binding preferences would provide

valuable insights into the functional diversity of PHD finger domains as epigenetic readers.

Author's response: we thank the reviewer for this suggestion. We have studied many PHD fingers, including ING2 (Nature 2006), and reported the differences in binding of PHD fingers to H3 and H3K4me3 in several reviews and research papers from our groups (including most recent TiBS 2023 and JBC 2023), and therefore would suggest avoiding repetition of this analysis in the current manuscript. We have added the following sentence in Fig. 3a legend: "For comparative analysis of PHD and PZP see refs.^{41,42}."

Reviewer 3, Comment 4: The manuscript would be substantially enhanced by the inclusion of a comprehensive model that illustrates the intricate interaction between the PZP domain and the

nucleosome. Such a model would greatly aid in visualizing the structural and functional aspects of this complex interaction.

Author's response: we agree, the model is now shown in Fig. 5h.

Reviewer 3, Comment 5: Have the authors assigned the resonances of Fig 2f that produced the K_d in Fig 2g? Similarly, Figs 4j and 4i? The chemical shift changes are so big whereas the higher concentrations induced tiny chemical shift changes due to slow exchange/tight binding. I wonder how the authors were able to trace the perturbed signals. Please provide more details. Again, I think this is a very strong/interesting manuscript. With proper revision, the manuscript will significantly contribute to the advancement of knowledge in several fields especially in the chromatin field.

Author's response: the binding curves shown in Fig. 2g are derived from tryptophan fluorescence assays, not NMR. We used only one HSQC titration experiment (shown in Fig. 2h) to calculate K_d for a very weak complex of JADE1_{PZP}-H3K4me3 (Fig. 2i) and updated NMR method section to include equation. All other K_d s were measured by fluorescence. We did not assign the PZP domain resonances.

Decision Letter, first revision:

Message: Our ref: NSMB-A47596A

14th Sep 2023

Dear Dr. Kutateladze,

Thank you for submitting your revised manuscript "Guiding the HBO1 complex function through the JADE subunit" (NSMB-A47596A). It has now been seen by the original referees and their comments are below. The reviewers find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Structural & Molecular Biology, pending minor revisions to satisfy the referees' final requests, particularly to mention the caveat that though JADE/HBO1 associates with MLL on chromatin, they may not be direct components of a soluble MLL complex, given the ChIP data, as per the request of reviewer #2, and to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements in 2-3 weeks. Please do not upload the final materials and make any revisions until you receive this additional information from us.

To facilitate our work at this stage, it is important that we have a copy of the main text as a word file. If you could please send along a word version of this file as soon as possible, we would greatly appreciate it; please make sure to copy the NSMB account (cc'ed above).

Thank you again for your interest in Nature Structural & Molecular Biology Please do not hesitate to contact me if you have any questions.

Sincerely,
Sara

Sara Osman, Ph.D.
Associate Editor
Nature Structural & Molecular Biology

Reviewer #1 (Remarks to the Author):

The authors have answered all my previous questions. The revised manuscript is significantly improved, and is recommended for publication in NSMB.

Reviewer #2 (Remarks to the Author):

In the revision, the authors have provided new evidence that JADE/HBO1 associates with MLL complex components such as Menin using fanChIP (Figure S1a,b). The Menin IP data in Figure S1a is quite weak and does not appear to be stoichiometric (ie. one would expect more Menin in the fJADE2 lane for example, as there is an increase in the amount of MLLN/C pulled down in that lane). The evidence of an interaction with Menin is better in the S1b, although the interaction with MLL1 is quite weak.

In addition, I asked if endogenous JADE proteins can interact with endogenous MLL complexes, and the authors used fanChIP to answer this question. The issue with fanChIP is that it can include complexes that are closely associated on chromatin rather than necessarily interacting directly as a soluble core complex, thus fanChIP is not an appropriate method for measuring endogenous protein complex interactions. The authors should have performed IP's with MLL and JADE3 antibodies on purified nuclear extracts, without DNA present. In particular, when the authors in the introduction say "We expressed FLAG-tagged JADE1, JADE2 and JADE3 individually in HEK293T cells and immunoprecipitated JADEs..." they need to make it clear that this is not a standard immunoprecipitation experiments with purified protein extracts.

I think the authors need to back away a little bit from their conclusions here. They have evidence that JADE/HBO1 associates with MLL on chromatin, but they should add the caveat that it may not be direct components of a soluble MLL complex, which is likely why the fanChIP contains such a small amount of Menin. They should also make it clear in the results that the JADE/HBO1-MLL interaction likely requires chromatin (or DNA) as an intermediary, and that this interaction could be a subset of MLL chromatin bound sites. This conclusion actually matches the ChIP-seq data nicely, as you can see even in the tracks in Figure 1e, MLL1 and JADE3 enrichment is in similar regions, but the profiles don't overlap like one would expect from components of the same protein complex (for example

JADE3 is more enriched at HOXA7 while MLL1-N is more enriched at HOXA5/HOXA-AS3).

Just to be clear, I don't think this takes away from the main conclusions of the paper which are still quite interesting, but I think it is important to acknowledge this point as it potentially tells us something about complex formation and activity.

The UCSC link provided in the reporting summary now gave me access to the session, I realized when I extracted it from the reporting session originally it created a typo. I apologize for the error. I had a look at the ChIP-seq data, it is very nice, but it also underscores the point above that although MLL/JADE/HBO1 bind to many similar regions, the shape of the peaks are also different in many places.

Other than the point about the MLL/JADE complex interactions, the authors have answered my questions and this remains a very interesting paper.

Reviewer #3 (Remarks to the Author):

I am satisfied with the authors' responses and revision.

Author Rebuttal, first revision:

Reviewer #2 (Remarks to the Author):

In the revision, the authors have provided new evidence that JADE/HBO1 associates with MLL complex components such as Menin using fanChIP (Figure S1a,b). The Menin IP data in Figure S1a is quite weak and does not appear to be stoichiometric (ie. one would expect more Menin in the fJADE2 lane for example, as there is an increase in the amount of MLLN/C pulled down in that lane). The evidence of an interaction with Menin is better in the S1b, although the interaction with MLL1 is quite weak.

In addition, I asked if endogenous JADE proteins can interact with endogenous MLL complexes, and the authors used fanChIP to answer this question. The issue with fanChIP is that it can include complexes that are closely associated on chromatin rather than necessarily interacting directly as a soluble core complex, thus fanChIP is not an appropriate method for measuring endogenous protein complex interactions. The authors should have performed IP's with MLL and JADE3 antibodies on purified nuclear extracts, without DNA present. In particular, when the authors in the introduction say "We expressed FLAG-tagged JADE1, JADE2 and JADE3 individually in HEK293T cells and immunoprecipitated JADEs..." they need to make it clear that this is not a standard immunoprecipitation experiments with purified protein extracts.

I think the authors need to back away a little bit from their conclusions here. They have

evidence that JADE/HBO1 associates with MLL on chromatin, but they should add the caveat that it may not be direct components of a soluble MLL complex, which is likely why the fanChIP contains such a small amount of Menin. They should also make it clear in the results that the JADE/HBO1-MLL interaction likely requires chromatin (or DNA) as an intermediary, and that this interaction could be a subset of MLL chromatin bound sites. This conclusion actually matches the ChIP-seq data nicely, as you can see even in the tracks in Figure 1e, MLL1 and JADE3 enrichment is in similar regions, but the profiles don't overlap like one would expect from components of the same protein complex (for example JADE3 is more enriched at HOXA7 while MLL1-N is more enriched at HOXA5/HOXA-AS3).

Just to be clear, I don't think this takes away from the main conclusions of the paper which are still quite interesting, but I think it is important to acknowledge this point as it potentially tells us something about complex formation and activity.

The UCSC link provided in the reporting summary now gave me access to the session, I realized when I extracted it from the reporting session originally it created a typo. I apologize for the error. I had a look at the ChIP-seq data, it is very nice, but it also underscores the point above that although MLL/JADE/HBO1 bind to many similar regions, the shape of the peaks are also different in many places.

Other than the point about the MLL/JADE complex interactions, the authors have answered my questions and this remains a very interesting paper.

Author's response: we thank Reviewer 2 for the insightful comments and toned down the MLL/HBO1 association, as suggested:

1. Suppl. Fig. 1 legend has been revised to include:
'Note that although components of the JADE/HBO1 complex associate with MLL1 on chromatin, they may not be direct components of a soluble MLL1 complex, as fanChIP assays capture protein-chromatin complexes.'
2. The main text, page 4: The following sentence has been removed (it was added in resubmission in response to this reviewer's original comment, but is not needed here):
'In further support, endogenous JADE3 was found to associate with endogenous HBO1, MLL1 and MENIN, a component of the MLL1 complex (Suppl. Fig. 1b).'

Final Decision Letter:

Message: 12th Feb 2024

Dear Dr. Kutateladze,

We are now happy to accept your revised paper "Guiding the HBO1 complex function through the JADE subunit" for publication as a Article in Nature Structural & Molecular Biology.

Acceptance is conditional on the manuscript's not being published elsewhere and on there being no announcement of this work to the newspapers, magazines, radio or television until the publication date in Nature Structural & Molecular Biology.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Structural & Molecular Biology style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

After the grant of rights is completed, you will receive a link to your electronic proof via email with a request to make any corrections within 48 hours. If, when you receive your proof, you cannot meet this deadline, please inform us at rjsproduction@springernature.com immediately.

You will not receive your proofs until the publishing agreement has been received through our system.

Due to the importance of these deadlines, we ask that you please let us know now whether you will be difficult to contact over the next month. If this is the case, we ask you provide us with the contact information (email, phone and fax) of someone who will be able to check the proofs on your behalf, and who will be available to address any last-minute problems.

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