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Crystal Structure of the N-terminal region of Human Ash2L Reveals a Winged Helix Motif Involved in DNA Binding

Yong Chen, Bingbing Wan, Kevin C Wang, Fang Cao, Yuting Yang, Angeline Protacio, Yali Dou, Howard Y Chang, Ming Lei

Corresponding author: Ming Lei, Univ. of Michigan

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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. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 21 December 2010

Thank you for the submission of your research manuscript to EMBO reports. We have now received the enclosed referee reports on it.

As you will see, while all the referees acknowledge that the study is potentially interesting and that the identification of a DNA binding motif in Ash2L is unexpected and important, they also raise a few concerns that need to be addressed before the manuscript can be considered for publication in EMBO reports.

All referees point out that it is still possible that Ash2L binds to DNA in a sequence-specific manner, and this possibility needs to be discussed. Referee 3 further indicates that the hypothesis that the Ash2LCTD promotes DNA binding of Ash2LNTD needs to be supported by experimental data. While we would also certainly welcome additional functional evidence that DNA binding of Ash2L is important for targeting active MLL complexes, this would not be an absolute requirement for the publication of your manuscript in our journal.

Going through the manuscript myself, I noticed that the figure legend for figure 4C does not explain the identity of the error bars that needs to be included. It also looks as if the gel in figure 3C is spliced together. Please provide the original gels used to prepare this figure and please re-run all the samples on the same gel and use this gel as new panel for figure 3C. Spliced bands of gels need to be clearly marked by a black or white line or sufficient white space to indicate gel splicing.

Given these evaluations and the constructive referee comments, I would like to give you the

opportunity to revise your manuscript, with the understanding that the referee concerns must be fully addressed and their suggestions (as detailed above and in their reports) taken on board. Acceptance of the manuscript will depend on a positive outcome of a second round of review and I should also remind you that it is EMBO reports policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

I look forward to seeing a revised version of your manuscript when it is ready.

Yours sincerely

Editor
EMBO Reports

REFEREE REPORTS:

Referee #1:

The mechanisms controlling the targeting of histone modification enzymes underpin the epigenetic control of gene expression and are therefore an important area of active research. The authors describe a crystal structure for the amino-terminal region of the H3K4 methyltransferase MLL complex protein Ash2L. This reveals both a PHD finger domain with an unusual arrangement and a winged helix domain, which leads to the description of a previously undescribed DNA binding function for Ash2L.

The structure presented is of reasonably high resolution and is supported with consistent crystallographic statistics.

The comparison of the Ash2L PHD finger with that of the PHF8 protein is useful and is a valid means of interpreting the significance of this region of the structure. The salient feature is the occupation of the binding site by the Ash2L Arg14 side chain. The text suggests that PHD fingers are exclusively readers of methylation, this is incorrect, and the authors should note that there are examples of unmodified lysine binding (eg. PHD finger of BHSC80, Lane et al 2007). The proposed hypothesis that the presence of Arg14 in this site and the packing of a loop to this region of the structure blocking other interactions is reasonable, but nevertheless the current structure does not preclude the possibility that histone binding could occur by displacement of these elements. In the absence of further biochemical evidence the authors should acknowledge this possibility. A figure showing the electron density for the arginine in this pocket should be provided along with details of whether the B factors for this loop region could indicate flexibility.

The discovery of the winged helix motif in the N-terminus of Ash2l suggests a hitherto unexpected function and is a significant finding. Supporting biochemical evidence is provided in the form of DNA mobility shift assays with a "random" DNA sequence. The measured affinity is weak and the increased affinity for full-length protein, or if the c-terminal domain is added in trans, suggests that further interactions beyond the current structure exist. Additionally the authors do not address the possibility that there may be a sequence specific element to the interaction of Ash2L with DNA and assume throughout that DNA binding is non-specific and relatively weak. Whilst addressing this issue may be beyond the scope of the current manuscript they should acknowledge that in the absence of data on specificity and the indicated broader interactions of full-length protein with DNA remaining uncharacterised this issue is still open.

The functional evidence supporting the importance of the DNA interaction in vivo is provided in the form of localisation of overexpressed wild-type or mutant Ash2L on the HoxC8 locus and a significant reduction is observed. A weakness of this approach is that it does not indicate if the DNA binding function is important for active assembled MLL complexes and therefore the case for the significance of this finding in targeting is not conclusively made which would have given the manuscript further significance. This should be acknowledged.

Minor Revisions

The title should be modified to indicate that the structure is of the N-terminal region of Ash2L

Figures 2 and 3A. The color scheme of the overlayed structures (shades of green) make interpretation difficult. The figures should be redrawn with more contrasting colors.

Figure 2C. The use of the same color scheme for the stick representation of the histone lysine chain and the Ash2L arginine is not clear. Use more contrasting colors.

Referee #2:

This is an interestiung paper that describes a surprising structural result: The Ash2 component of the MLL family of histone methyltransferases contains a DNA-binding domain structure. This is important because a big question in the study of this family of epigenetic regulators is both how they are targeted to specific promoters and how they are maintained there.

I believe that the manuscript can be accepted essentially as is. I have only two comments:

1/ I appreciate that the authors have only shown that the Winged Helix motif has non-specific DNA binding activity, but why could it not possess DNA-binding specificity? For example, the CpG recognition motif called CXXC found in MLL and the Cfp1 component of the Set1 complex has DNA-binding (albeit limited) specificity.

2/ I would have found it interesting in the discussion for the authors to relate their story to the CXXC motif as it shows that the MLL-related histonemethyl transferases have multiple possible points of contact with the DNA.

Referee #3:

This paper describes the crystal structure of the Ash2L N-terminal domain which consists of a PhD domain and an unexpected winged helix domain. The winged helix domain has a positive surface which is suggestive of DNA binding in analogy to other winged helix domains.This is an important finding since it sheds light on the mechanism of action/target for MLL complexes.

Specific comments:
The section on DNA binding of Ash2L needs significant attention. It is shown that the Ash2LNTD binds non-specifically to random DNA with a Kd of 12 uM. This is compared to Fox01 which binds to a high affinity specific DNA binding site with a Kd of 12 nM. This means that the binding of Ash2LNTD to DNA is 1000x weaker than FoxO1 not 100x as stated. The Kd for Ash2L is also compared to that of FoxM1 which has a Kd of 7 uM for the classical forkhead consensus site. The authors need to comment on whether they think that Ash2L is a sequence specific DNA binding protein for which they have not found the specific site or a non-specific DNA binding protein which is part of a chromatin methylation complex.

It is proposed that the Ash2LCTD acts synergistically to promote DNA binding of the Ash2LNTD. The authors do not explain how they think this may occur since they say that the Ash2LCTD does not bind to DNA and does not bind directly to the Ash2LNTD. The Ash2LCTD may interact with both Ash2LNTD and DNA in the presence of DNA. The authors should perform some experiments to address this question.

1st Revision - authors' response 11 April 2011

Response to Editor's comments

All referees point out that it is still possible that Ash2L binds to DNA in a sequence-specific manner, and this possibility needs to be discussed.

To address the DNA-binding specificity of Ash2L, we have carried out an in vitro library-screening assay. After several rounds of selection, we did not get any consensus DNA-binding sequence for Ash2L. Furthermore, our Chip-chip data also showed a broad distribution of Ash2L across the HOXA locus, suggesting that $Ash2L_{WH}$ binds to DNA with no strong sequence specificity. We understand that, despite these new data, we still cannot rule out the possibility that Ash2L has a weak DNA sequence specificity. We discussed this possibility in the revised manuscript.

Referee 3 further indicates that the hypothesis that the Ash2LCTD promotes DNA binding of Ash2LNTD needs to be supported by experimental data.

We have performed additional experiments to test if $Ash2L_{CTD}$ might interact with $Ash2L_{NTD}$ in the presence of DNA. However, both ITC and in vitro GST-pull-down assays failed to detect any interaction between $Ash2L_{NTD}$ and $Ash2L_{CTD}$ (Supplementary Fig. S4). Therefore, based on the current data, we cannot explain why full-length Ash2L binds to DNA better than $Ash2L_{NTD}$. The structure study of the full length Ash2L and its interaction DNA are needed to fully understand the underlying mechanism. We are working on this project right now. Hopefully, we can provide the structure of the Ash2L-DNA complex in the future.

While we would also certainly welcome additional functional evidence that DNA binding of Ash2L is important for targeting active MLL complexes, this would not be an absolute requirement for the publication of your manuscript in our journal.

In the revised manuscript, our new Chip-chip data demonstrated that the DNA binding activity plays a key role in targeting Ash2L to the HoxA locus (Fig. 4C). Strikingly, we also observed that the K131A mutation of Ash2L overcomes the chromatin domain boundary set up by chromosomal looping and histone marks and allows Ash2L to spread into otherwise silent chromatin domains (Fig. 4C). Therefore, it is possible that Ash2L also plays a role in chromatin domain demarcation.

Going through the manuscript myself, I noticed that the figure legend for figure 4C does not explain the identity of the error bars that needs to be included.

The error bar is the s.d. from triplicate experiments. We have included the explanation of the error bars in Figure legend of supplementary Figure S5B.

It also looks as if the gel in figure 3C is spliced together. Please provide the original gels used to prepare this figure and please re-run all the samples on the same gel and use this gel as new panel for figure 3C. Spliced bands of gels need to be clearly marked by a black or white line or sufficient white space to indicate gel splicing.

We didn't manipulate the gel image (such as splicing) except the brightness adjustment. Figure 3C is the gel image from the PhosphorImager system. The white band you saw in the image was from the malfunction of Typhoon scanner on the day we performed the experiment. Since we repeated this gel-shift experiment several times, we now include the image from another experiment as new Figure 3C, to avoid misinterpretation.

Chen et al., "Crystal Structure of the N-terminal region of Human Ash2L Reveals a Winged Helix Motif Involved in DNA Binding"

Reviewer 1

The mechanisms controlling the targeting of histone modification enzymes underpin the epigenetic control of gene expression and are therefore an important area of active research. The authors describe a crystal structure for the amino-terminal region of the H3K4 methyltransferase MLL complex protein Ash2L. This reveals both a PHD finger domain with an unusual arrangement and a winged helix domain, which leads to the description of a previously undescribed DNA binding function for Ash2L.

The structure presented is of reasonably high resolution and is supported with consistent crystallographic statistics.

Thank you.

The comparison of the Ash2L PHD finger with that of the PHF8 protein is useful and is a valid means of interpreting the significance of this region of the structure. The salient feature is the occupation of the binding site by the Ash2L Arg14 side chain. The text suggests that PHD fingers are exclusively readers of methylation, this is incorrect, and the authors should note that there are examples of unmodified lysine binding (eg. PHD finger of BHSC80, Lane et al 2007).

We thank the reviewer for pointing out this error. We have reworded the manuscript accordingly.

The proposed hypothesis that the presence of Arg14 in this site and the packing of a loop to this region of the structure blocking other interactions is reasonable, but nevertheless the current structure does not preclude the possibility that histone binding could occur by displacement of these elements. In the absence of further biochemical evidence the authors should acknowledge this possibility. A figure showing the electron density for the arginine in this pocket should be provided along with details of whether the B factors for this loop region could indicate flexibility.

We understand that our structure presented here only shows Ash2L$_{NTD}$ itself. It is possible that other factors could bind to Ash2L to open up the pocket for histone peptide binding. We discussed this possibility in the revised manuscript. Following this reviewers' suggestion, we now showed the electron density for Arg14 in Supplementary Figure S3A. The average B factors for loop L$_{BC}$ is 41.5 Å^2 that is comparable to the overall B-factor for the whole structure (44.7 Å^2), indicating that this loop is a rigid region in the structure. An overall structure colored by B factors is now presented in the Supplementary Figure S3B.

The discovery of the winged helix motif in the N-terminus of Ash2l suggests a hitherto unexpected function and is a significant finding.

Thanks.

Supporting biochemical evidence is provided in the form of DNA mobility shift assays with a "random" DNA sequence. The measured affinity is weak and the increased affinity for full-length protein, or if the c-terminal domain is added in trans, suggests that further interactions beyond the current structure exist. Additionally the authors do not address the possibility that there may be a sequence specific element to the interaction of Ash2L with DNA and assume throughout that DNA binding is non-specific and relatively weak. Whilst addressing this issue may be beyond the scope of the current manuscript they should acknowledge that in the absence of data on specificity and the indicated broader interactions of full-length protein with DNA remaining uncharacterised this issue is still open.

We have performed additional experiments to test if $Ash2L_{CTD}$ might interact with $Ash2L_{NTD}$ in the presence of DNA. However, both ITC and in vitro GST-pull-down assays failed to detect any interaction between $Ash2L_{NTD}$ and $Ash2L_{CTD}$ (Supplementary Figure S4). Therefore, based on the current data, we cannot explain why full-length Ash2L binds to DNA better than $Ash2L_{NTD}$. The structure study of the full length Ash2L and its interaction DNA are needed to fully understand the underlying mechanism. We are working on this project right now. Hopefully, we can provide the structure of the Ash2L-DNA complex in the future.

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We agree with the reviewer that the current data did show the direction connection between the DNA binding function of Ash2L and the chromosome targeting of the MLL complex. Our data only demonstrated that the DNA binding activity of Ash2L plays a key role in targeting Ash2L itself to the Hox locus. We have added a discussion section about this point in the revised manuscript. The recruitment of the MLL complexes to their target genes involves a combination of different mechanisms. Recent studies suggested that MLL interactions with menin and LEDGF play a key role in targeting MLL complexes to

the HOX locus. In contrast, the CXXC motif in MLL is dispensable for this localization. Instead, MLL_{CXXC} binds to nonmethylated CpG DNA sites that are essential for maintenance of appropriate epigenetic marks and continued transcription. As Ash2L is a common core component of all MLL complexes, we propose that Ash2L provides a non-specific DNA-binding activity that collaborates other gene-specific mechanisms to stabilize the association of MLL complexes with active chromatin domains. Notably, we also demonstrated that a single mutation in the WH motif of Ash2L (K131A) overcomes the chromatin domain boundary set up by chromosomal looping and histone marks and allows Ash2L to spread into otherwise silent chromatin domains. Thus, it is possible that Ash2L plays a role in chromatin domain demarcation.

Minor Revisions

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We have revised the title as suggested.

Figures 2 and 3A. The color scheme of the overlayed structures (shades of green) make interpretation difficult. The figures should be redrawn with more contrasting colors.

We have changed color as suggested to have better visualization.

Figure 2C. The use of the same color scheme for the stick representation of the histone lysine chain and the Ash2L arginine is not clear. Use more contrasting colors.

We have corrected this in the revised manuscript.

Reviewer 2

This is an interesting paper that describes a surprising structural result: The Ash2 component of the MLL family of histone methyltransferases contains a DNA-binding domain structure. This is important because a big question in the study of this family of epigenetic regulators is both how they are targeted to specific promoters and how they are maintained there.

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Specific comments:

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2nd Editorial Decision 02 May 2011

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. As you will see from the referee comment pasted below, two minor changes still have to be made to the manuscript text. I suggest that you send us the new sentence for page 7 via email and we will replace the existing one for you. I also could not find the accession number/code for the new structure you describe in the manuscript. Please let us know as soon as possible what the accession number is so that we can proceed with the export of the manuscript to the npg production team.

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.

Yours sincerely,

Editor
EMBO Reports

REFEREE REPORTS:

Referee #3

In the revised manuscript the authors have were possible addressed the issues raised by all three referees. Where experimental evidence has not fully resolved an issue (for example whether Ash2L has any DNA sequence specificity) the revised manuscript provides sufficient caveats such that a balanced argument is proposed. I recommend publication of this version of the manuscript.

Minor typographical errors.
page 7: para 2: line 10. sentence needs rewording.
page 8: para 1: line 9. should be "presence"

2nd Revision - authors' response 02 May 2011

Thanks for handling our paper. Here are the information you asked.

PDB ID code: 3RSN

The revised sentence on Page 7:
This relatively low DNA-binding affinity can be explained by the structural difference between Ash2L and FoxO1. Wing 2 in FoxO1 plays an important role in DNA binding, as deletion of this loop completely abolished the interaction between FoxO1 and DNA (Brent et al., 2008).

