Histone H1 binding is inhibited by histone variant H3.3

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Review timeline:
- Submission date: 06 July 2009
- Editorial Decision: 14 August 2009
- Revision received: 07 September 2009
- Accepted: 07 September 2009

Transaction Report:
(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 14 August 2009

Thank you for submitting your manuscript for consideration by The EMBO Journal. Three referees have now evaluated your manuscript and I enclose their reports below. As you will see from their comments the referees express interest in the anticorrelation between H1 and H3.3 occupancy and recommend publication in EMBO J pending some revision. This includes changes to the figures and if possible some further experiments to gain some further support for the relationship, such as using the null H3.3 mutant or if H1 depletion affects H3.3 binding. Should you be able to address these issues, we would be happy to consider a revised manuscript.

I should remind you that it is EMBO Journal 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. When you submit a revised version to the EMBO Journal, please make sure you upload a letter of response to the referees' comments.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1 (Remarks to the Author):

The manuscript describes the authors characterization of the genome-wide distribution of histone H1
in Drosophila using DamID. Interestingly, the authors found that H1 binding is reduced around the transcription start sites of active genes and potential regulatory elements. This reduction extends beyond nucleosome-free regions, suggesting that it is not a trivial consequence of nucleosome loss. By contrast, the authors identified a strong negative correlation between H1 binding and levels of the histone variant H3.3, suggesting that H3.3 incorporation antagonizes H1 binding. Consistent with this possibility, the authors found that the partial knockdown of H3.3 leads to increased H1 binding and a change in nucleosome-repeat length. Based on these observations, the authors conclude that H3.3 incorporation negatively regulates the binding of H1 to chromatin.

Although many of the effects reported in this manuscript are relatively subtle, the technical quality of the work is high and support the authors' conclusions about the genomic distribution of H1 and its relationship to H3.3 occupancy. The authors' hypothesis that H3.3 incorporation antagonizes H1 binding is exciting and raises many interesting questions about the role of histone variants in the regulation of higher-order chromatin structure. This work will certainly be of interest to a wide audience.

Although my enthusiasm for this manuscript is high, several minor issues should be addressed prior to publication:

1. The authors do not clearly explain why they expect to see a change in NRL following H3.3 knockdown. A relatively small percentage of the genome exhibits increased H3.3 and reduced H1 binding. Do the authors believe that the relatively subtle changes in H1 occupancy in these regions resulting from H3.3 knockdown could cause global changes in NRL? Alternatively, do the authors believe that H3.3 influences H1 binding in other regions of the genome? The authors need to clarify their models and expectations.

2. The authors should expand their discussion of the potential mechanisms by which H3.3 incorporation reduces H1 binding. One straightforward possibility is that the replication-independent incorporation of H3.3 displaces H1. The authors do not discuss this possibility. Instead, they seem to favor the idea that H1 has a lower affinity for chromatin containing H3.3. Given the intense interest in replication-independent H3.3 assembly and dynamic H1 exchange, this issue merits further discussion.

Other minor comments:

1. Title page: change "Standford" to "Stanford".

3. page 3, first paragraph: the authors should cite the following references to support their statement that decreased H1 expression causes chromosome decondensation in flies: Lu et al., (2009) and Siriaco et al., (2009)


Referee #2 (Remarks to the Author):

The manuscript by Braunschweig et al. describes mapping of linker histone H1 in the fruit fly genome using the DamID method. The authors find that H1 binds evenly to most of the genome, with the exclusion of a large number of localized domains. H1 exclusion correlates with the start sites of genes, as well as with potential regulatory elements, such as binding sites of Polycomb group proteins. H1 exclusion strongly anticorrelates with histone H3.3, which was shown to be enriched at active genes and potential regulatory sequences in earlier studies. RNAi knockdown of H3.3 leads to enhanced H1 binding to normally depleted sites. This is a well-executed study addressing an important and understudied aspect of chromatin biology. The conclusions are significant and well-justified, and will be of broad interest. The manuscript is generally well-written and clear, but the figures could use some improvement in my opinion, as described below. I feel that the manuscript is suitable for publication in EMBO, and have only relatively minor suggestions for improvement.

1. Figure panels are frequently cited out of order; sometimes a panel isn't cited until a later figure
has been. I found this confusing, and suggest reordering the figures (or the text) so that they are in sync.

2. At the end of the second paragraph on page 6, the authors cite figure 2, but some of the relevant data are in supplementary figure 2.

3. In the third paragraph on page 6, the authors state that RpII18 is strongly anticorrelated with H1, but only a qualitative figure panel representing a very small part of the genome is provided in support. I suggest including a correlation coefficient and a scatter plot. A scatter plot would be similarly helpful to visualize the anticorrelation between H3.3 and H1 (page 8).

4. Figure 3C is cited in the fourth paragraph on page 8; the appropriate panel is 2C.

5. I found the omission of RpII18 from panels C and D of figure 3 puzzling. The traces are included in panels A and B and I feel they should be present in all panels.

6. I suggest moving figure 5A to the supplement. A great deal of imagination is required to see the differences in nucleosome ladders in this panel (more than I apparently possess), and I wouldn't find this result at all convincing without the autocorrelation analysis.

Referee #3 (Remarks to the Author):

This manuscript presents genome-wide binding map of linker histone H1 in Drosophila cell culture. The authors demonstrate that H1 occupancy is reduced at transcription start sites and cis-regulatory elements of active genes and propose that it is inversely correlated with the occupancy of replacement histone variant H3.3.

Histone H1 binding profile was mapped with an ectopically expressed H1-DamID fusion protein. Figs. 1A and 1B illustrate profiles of H1 binding in relation to those of Pc and HP1 in a euchromatic region of 2L and at the heterochromatin-euchromatin boundary of 2R, respectively. H1 is present throughout the genome with thousands of characteristic "dips" of occupancy, several kb-long. Statistical analyses indicate that H1 binding (Fig. 1C) or density of H1 "dips" (Fig. 1D) do not strongly correlate with heterochromatin or Pc domains. However, H1 occupancy dips do correlate with higher occupancy of RNA polymerases and histone H3.3 (exemplified in Fig. 1E). The inverse correlation between H1 and H3.3 occupancies is statistically significant and observed over 5'- and 3'-ends of active Pol II genes, transposable elements, tRNA genes, trxG and PcG binding sites (Fig. 2).

In Fig. 3, the authors use FAIRE analysis to suggest that intergenic H1 dips may correlate with regulatory elements. H3.3 knockdown (Figs. 4A-C) causes mild but reproducible increase of H1 occupancy at typical H1 dips (Fig. 4D). This dependency appears more substantial at sites that normally contain the least amount of H3.3 (Fig. 4E). Figs. 4F-H further demonstrate that this dependency is statistically significant at H1 dips in 5'-ends of active genes and intergenic sites. Finally, a slight increase in genome-wide nucleosome repeat length is observed upon H3.3 knockdown (Fig. 5).

This manuscript reports results of interesting and timely studies. Previously, genome-wide localization of H1 has only been assessed by low-resolution methods, such as polytene chromosome IF staining, and did not provide sufficient details of H1 occupancy in relation to genomic features. In general, the work features very high technical quality of experiments and mathematical analyses of the data.

On the other hand, the major finding of the paper (H1 binding dependence on H3.3 presence in chromatin) is weakly substantiated. What is the mechanism of this correlation? Is it a result of direct physical interactions or is it mediated by secondary effects, such as regulation of transcriptional activity at genomic loci? Given the small magnitude of the observed effect of partial H3.3 knockdown in Kc167 cells, it is necessary to perform similar experiments in vivo with available null mutants of H3.3 genes (Hödl and Basler, 2009). To understand the casual relationship between H1 and H3.3 binding, it would also be interesting to see whether H1 knockdown reciprocally affects
H3.3 distribution in chromatin.

Referee #1:

[...]

1. The authors do not clearly explain why they expect to see a change in NRL following H3.3 knockdown. A relatively small percentage of the genome exhibits increased H3.3 and reduced H1 binding. Do the authors believe that the relatively subtle changes in H1 occupancy in these regions resulting from H3.3 knockdown could cause global changes in NRL? Alternatively, do the authors believe that H3.3 influences H1 binding in other regions of the genome? The authors need to clarify their models and expectations.

We tried to analyse different genomic regions (H1 dips vs. bulk, transcriptionally active vs. inactive regions) separately, but subsetting dataset leads to too much noise (i.e., 'wobbly' ACF) to estimate nucleosome repeat length accurately enough to detect differences.

- We have now addressed this in the Results (p.11) and briefly in the Discussion, p.12, second paragraph.

2. The authors should expand their discussion of the potential mechanisms by which H3.3 incorporation reduces H1 binding. One straightforward possibility is that the replication-independent incorporation of H3.3 displaces H1. The authors do not discuss this possibility. Instead, they seem to favor the idea that H1 has a lower affinity for chromatin containing H3.3. Given the intense interest in replication-independent H3.3 assembly and dynamic H1 exchange, this issue merits further discussion.

- This is an interesting suggestion, and we have extended the Discussion accordingly, p.13, second paragraph.

Other minor comments:

1. Title page: change "Standford" to "Stanford".

- changed

3. page 3, first paragraph: the authors should cite the following references to support their statement that decreased H1 expression causes chromosome decondensation in flies: Lu et al., (2009) and Siriaco et al., (2009)

- added references


- added reference
Referee #2:

[...]

1. Figure panels are frequently cited out of order; sometimes a panel isn’t cited until a later figure has been. I found this confusing, and suggest reordering the figures (or the text) so that they are in sync.

- reordered several figure panels: swapped 1A and 1B; swapped 2C and 2D; moved 3B to 3D; moved 4B,C to 4G,H; swapped supplementary figure S1A and B
- changed order of mention: fig. 4E before 2A,B; 2EF before 3

2. At the end of the second paragraph on page 6, the authors cite figure 2, but some of the relevant data are in supplementary figure 2.

- cited supplementary fig. 2A-D

3. In the third paragraph on page 6, the authors state that RpII18 is strongly anticorrelated with H1, but only a qualitative figure panel representing a very small part of the genome is provided in support. I suggest including a correlation coefficient and a scatter plot. A scatter plot would be similarly helpful to visualize the anticorrelation between H3.3 and H1 (page 8).

- added correlation coefficient and p-value
- added scatter plot of H1 vs. RpII18 and H3.3 as supplementary fig. 3

4. Figure 3C is cited in the fourth paragraph on page 8; the appropriate panel is 2C.

- changed

5. I found the omission of RpII18 from panels C and D of figure 3 puzzling. The traces are included in panels A and B and I feel they should be present in all panels.

- added traces for RpII18 to fig. 3C,D

6. I suggest moving figure 5A to the supplement. A great deal of imagination is required to see the differences in nucleosome ladders in this panel (more than I apparently possess), and I wouldn’t find this result at all convincing without the autocorrelation analysis.

- moved fig. 5A to supplementary fig. 5A and improved contrast

Referee #3:

[...] 

On the other hand, the major finding of the paper (H1 binding dependence on H3.3 presence in chromatin) is weakly substantiated. What is the mechanism of this correlation? Is it a result of direct physical interactions or is it mediated by secondary effects, such as regulation of transcriptional activity at genomic loci? Given the small magnitude of the observed effect of partial H3.3 knockdown in Kc167 cells, it is necessary to perform similar experiments in vivo with available null mutants of H3.3
genes (Hödl and Basler, 2009). To understand the causal relationship between H1 and H3.3 binding, it would also be interesting to see whether H1 knockdown reciprocally affects H3.3 distribution in chromatin.

- added to discussion, see Reviewer #1

We appreciate the value of the suggested experiments. However, we do not deem it feasible to conduct them in a reasonable time frame for the following reasons:

- Remapping H1 in vivo would require transgenic Dam-H1 flies that do currently not exist. Over the past few years we have encountered technical problems with the generation of certain Dam-fusion lines. We are in the process of designing an improved strategy, but this still needs to be tested and validated, and will take at least 6 months. Added to the time required to cross Dam-H1 lines to the H3.3 mutants (the two H3.3 genes are on different chromosomes), we feel that this would cause a delay that is unacceptable given the merely confirmatory nature of this experiment.

- As stated in the Discussion, we have previously tried to knock down H1 in Kc cells using various protocols, without success.

Additional changes:

- Fig. 4G,H (formerly 4B,C) have been replaced with clearer Western blots. A "crude lysate" lane has been added to fig.4H.

- added reference to supplementary fig. 4A (p.9, first paragraph)

- added primer sequences of amplicon (Fmo-2) used for normalization of expression analyses to supplementary table 1