Drosophila Histone Locus Body assembly and function involves multiple interactions

Kaitlin Koreski, Leila Rieder, Lyndsey McLain, Ashlesha Chaubal, William Marzluff, and Robert Duronio

Corresponding author(s): Robert Duronio, University of North Carolina

Review Timeline:

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|------------------------|------------|
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Editor-in-Chief: Matthew Welch

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.)
RE: Manuscript #E20-03-0176
TITLE: “Drosophila Histone Locus Body assembly and function involves multiple interactions”

Michael-

you will see from their comments that both referee find your manuscript of interest. While referee 2 has no further queries, referee 1 is asking for several pieces of information mostly to better characterize the model system; these seem reasonable requests considering the central role of the model system for your conclusions. I am looking forward to these revisions which we will be glad to consider.

Sincerely,

Tom Misteli
Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Duronio,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor’s decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor’s and reviewers’ comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you have opted out of publishing the review history.

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To submit the cover letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

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Reviewer #1 (Remarks to the Author):

In this manuscript, Koreski and coauthors focus on the functional characterization of genetic elements responsible for the formation of the Histone Locus Body (HLB) in Drosophila. This prominent nuclear structure forms at replication-dependent (RD) histone gene loci and concentrates factors essential for histone pre-mRNA biosynthesis. The authors provide evidence that the
A transgenic array containing 12 copies of the histone repeat unit can functionally complement the loss of the ~200 endogenous RD histone genes and rescue embryonic lethality. Previously, the authors demonstrated that the Drosophila histone H3-H4 promoter is required for HLB formation (Salzler et al., 2013). Interestingly, here they showed that the replacement of all H2a-H2b promoters (they do not have the conserved GAGA repeat elements which specifically target CLAMP protein) with H3-H4 promoters in the transgenic 12x histone H2a-H2b repeat unit induces the ectopic HLB assembly at the array, in contrast to the array with H2a-H2b promoters. Furthermore, the newly formed HLB assembled on the mutant 12x array in the absence of endogenous RD histone genes contains all known factors present in the wild type HLB, including CLAMP. The authors conclude that HLB formation is driven by multiple protein-protein and/or protein-DNA interactions and that subsequent transcriptional activation of histone genes is critical for HLB growth and maturation.

This is an interesting and straightforward manuscript that explores principles not fully understood, which drive the formation of biomolecular condensates by liquid-liquid phase separation. The experiments are generally well designed and data are novel. Specific comments and questions are below.

The authors should better characterize functionally CLAMP protein in the HLB formed on the transgene array, even though all of the known CLAMP binding sites from the histone repeat. Does CLAMP exhibit different kinetics or binding affinity in ectopic HLB formed on 12xPR array in the absence of HisC than it does in wild type HLB? Is CLAMP localized differently in ectopic HLBs than in endogenous HLBs? The authors should check whether the ectopic HLB can assemble on a 12xPR array in the CLAMP-depleted embryos.

Is there a link between the transcriptional output of histone gene arrays used in this study and HLB size? The presented HLBs appear to be slightly heterogeneous in size. The authors should quantify and compare the relative sizes of studied HLBs. Perhaps larger or smaller HLBs exhibit different behaviors. Is there a chance that they may also represent HLBs at different maturation stages, with differing compositions? Perhaps these features significantly influence their fate, behavior and overall intra-nuclear dynamics.

Please, explain more explicitly how a single histone repeat unit can stimulate HLB formation. Is this HLB fully comparable to HLBs formed under different experimental conditions?

The authors do not comment on the implications of their data. What is the benefit of rescuing the loss of endogenous RD histone genes by much shorter transgene arrays with different gene expression? How does the presence of a single wild-type histone gene in the transgene 12x array affect the expression of neighboring genes than in the normal 12xPR array?

Reviewer #2 (Remarks to the Author):

The most prominent nuclear organelle in most eukaryotic cells is the nucleolus, which assemble at the locus of the ribosomal RNA genes. A less prominent, but probably ubiquitous nuclear organelle, is the histone locus body (HLB), which functions in the processing of histone pre-mRNA. In this manuscript the authors examine the HLBs of Drosophila using a variety of genetic and cytological techniques.

The Drosophila genome contains a single cluster of about 100 repeats of the five histone genes, and the HLBs are associated with this cluster. Deletion of the endogenous histone genes is lethal, but the authors show that this lethality can be rescued by a transgenic array consisting of only 12 copies of the histone gene cluster. In a set of well-documented and unusually complete experiments, the authors show that the HLBs formed on this cluster contain all the known factors that normally bind there.

Although straightforward in conception, this series of experiments required a thorough knowledge of Drosophila genetics and considerable skill in both genetic manipulation and cytological analysis. The manuscript is well-written and relatively easy to follow, despite the complex genetic and cytological manipulations described by the authors. I feel that it is an important contribution to our understanding of the HLB. Whereas much of our previous information about this important nuclear organelle comes from organisms without robust genetics, this analysis in Drosophila includes experiments that are difficult or impossible in other organisms.
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In this manuscript, Koreski and coauthors focus on the functional characterization of genetic elements responsible for the formation of the Histone Locus Body (HLB) in Drosophila. This prominent nuclear structure forms at replication-dependent (RD) histone gene loci and concentrates factors essential for histone pre-mRNA biosynthesis. The authors provide evidence that the transgenic array containing 12 copies of the histone repeat unit can functionally complement the loss of the ~200 endogenous RD histone genes and rescue embryonic lethality. Previously the authors demonstrated that the Drosophila histone H3-H4 promoter is required for HLB formation (Salzler et al., 2013). Interestingly, here they showed that the replacement of all H2a-H2b promoters (they do not have the conserved GAGA repeat elements which specifically target CLAMP protein) with H3-H4 promoters in the transgenic 12x histone H2a-H2b repeat unit induces the ectopic HLB assembly at the array, in contrast to the array with H2a-H2b promoters. Furthermore, the newly formed HLB assembled on the mutant 12x array in the absence of endogenous RD histone genes contains all known factors present in the wild type HLB, including CLAMP. The authors conclude that HLB formation is driven by multiple protein-protein and/or protein-DNA interactions and that subsequent transcriptional activation of histone genes is critical for HLB growth and maturation.

This is an interesting and straightforward manuscript that explores principles not fully understood, which drive the formation of biomolecular condensates by liquid-liquid phase separation. The experiments are generally well designed and data are novel. Specific comments and questions are below.

We thank the reviewer for their appreciation of the work and for their helpful comments for how to improve the manuscript.

The authors should better characterize functionally CLAMP protein in the HLB formed on the transgene array, even though all of the known CLAMP binding sites from the histone repeat. Does CLAMP exhibit different kinetics or binding affinity in ectopic HLB formed on 12xPR array in the absence of HisC than it does in wild type HLB? Is CLAMP localized differently in ectopic HLBS than in endogenous HLBS? The authors should check whether the ectopic HLB can assemble on a 12xPR array in the CLAMP-depleted embryos.

To further our understanding of how CLAMP interacts with the 12xPR array, we performed ChIP seq analysis. We used fly strains in which the homozygous HisC deletion, which removes all endogenous replication dependent histone genes, was rescued with either homozygous 12xDWT control or homozygous 12xPR transgenes. We generated short read Illumina sequencing libraries from chromatin prepared from early embryos and immunoprecipitated with anti-CLAMP antibodies. These libraries were generated prior to the COVID-19 laboratory shutdown and submitted for sequencing while the paper was being reviewed. Thankfully we have been able to bioinformatically analyze the sequencing data during the shutdown. The results are presented in new Figure 4. We found that on the 12xDWT control transgene that CLAMP is strongly enriched at the H3-H4 bidirectional promoter, which contains GAGA repeats, as it is at the endogenous HisC locus (Rieder et al., 2017). This signal was completely lost from the 12xPR transgene in which all the H3-H4 promoters were replaced with H2a-H2b promoters, which lack GAGA.
repeats, and there was no CLAMP binding anywhere on the repeat unit. Because we observe robust HLB staining with anti-CLAMP antibodies of the 12xPR locus in the absence of the endogenous histone genes, these data strongly argue that CLAMP can be recruited to histone genes by protein-protein interactions at the histone locus in the absence of sequence specific DNA binding. This result supports our main conclusion that HLBs form by multiple protein-protein and protein-nucleic acid interactions, as has been observed for essentially all other biomolecular condensates.

Is there a link between the transcriptional output of histone gene arrays used in this study and HLB size? The presented HLBs appear to be slightly heterogeneous in size. The authors should quantify and compare the relative sizes of studied HLBs. Perhaps larger or smaller HLBs exhibit different behaviors. Is there a chance that they may also represent HLBs at different maturation stages, with differing compositions? Perhaps these features significantly influence their fate, behavior and overall intra-nuclear dynamics.

The question of if and how HLB size heterogeneity affects histone mRNA biosynthesis is very interesting. As the reviewer surmised, we previously showed that HLBs can exist in different stages of maturation, either in the early embryo when they are initially formed by a stepwise process as zygotic histone transcription begins (e.g. White et al., 2011), or after genetic manipulations that block transcription initiation from the H3-H4 promoter (e.g. “proto-HLBs” containing only Mxc and FLASH as described in Salzler et al., 2013).

We studied only the initial formation of the HLB’s in this paper in young syncytial blastoderm embryos, to determine whether there was any temporal difference in formation of the HLBs due to replacing the H3-H4 promoter. In these early nuclear cycles with short S phases, HLBs are first formed on the endogenous histone locus in cycle 11 and increase in size in subsequent cycles. The endogenous histone genes are transcribed starting when the HLB forms, but we don’t know the absolute level of expression of the genes, because of the large amount of maternal histone mRNA. Since the S phases are very short at this stage, only small amounts of zygotic histone mRNA could be made. Note that transcription during these cycles (i.e. 11-14) is not essential, since embryos containing no histone genes develop until cycle 15 at which time they arrest. We have added a paragraph to the Results section describing these points.

In addition, in our original submission of this work we included a paper that is nearly in press describing collaborative studies with Stefano Di Talia’s lab that address the connection between HLB size and function (Hur et al., 2019; BioRXiv https://doi.org/10.1101/789933). We found a strong correlation between HLB size and the amount of nascent histone transcripts in young syncytial blastoderm embryos. A great deal more work is necessary to conclusively determine if and how HLB size influences histone mRNA biosynthesis, and such studies are well beyond the scope of this paper. At several different places throughout the current manuscript we have referred to and described prior studies that address the important issues raised by the reviewer.

Please, explain more explicitly how a single histone repeat unit can stimulate HLB formation. Is
this HLB fully comparable to HLBs formed under different experimental conditions?

Our study of HLBs formed on single histone repeats was published in Salzler et al., 2013. These transgenes were able to stimulate readily visible HLB formation in polyplid salivary gland cells, but in diploid cells they were too small to detect. Nevertheless, we were able to detect histone gene expression from these single arrays in diploid cells from embryos. We concluded that transgenes containing a single histone repeat were functional. Moreover, we show in Hur et al., 2020 that the size of the HLB in embryos is dependent on the number of histone genes. Thus a single copy of the repeat unit likely forms a HLB, which would be smaller than the HLB formed on a 12x cluster. This HLB would contain multiple copies of Mxc and U7 snRNP. We can’t tell whether it is fully comparable to an HLB formed on a histone gene array, but it expresses histone mRNAs.

The authors do not comment on the implications of their data. What is the benefit of rescuing the loss of endogenous RD histone genes by much shorter transgene arrays with different gene expression? How does the presence of a single wild-type histone gene in the transgene 12x array affect the expression of neighboring genes than in the normal 12xPR array?

The main benefit of our system is that we can manipulate the genotype of the full complement of functional replication dependent histone genes, a capability that is not available in any other experimental animal system. Our ability to synthesize specific histone genotypes with short arrays that fully complement loss of the endogenous histone genes allows us to determine the cis acting elements required for HLB assembly and histone gene expression (this study), as well as to determine the function of specific histone tail residues that are post-translationally modified, as we have done in several prior studies over the past few years (PMID 30279224, 30700014, 28346137, 29133298, 29466941, 27566777, 25669886).

Thus, this is the only system where we can identify cis elements that are responsible for expression from the histone gene cluster in vivo. We are able to ask if changes in HLB assembly and/or histone gene expression levels have any effect on fly development. In fact, we were very surprised that the 12xPR could rescue the HisC deletion, especially since it was very poorly expressed in the presence of the endogenous histone genes. Our data using a 12x transgene in which one of the PR histone gene repeats was replaced with a wild type repeat (12xPR-1) indicates that one repeat in cis does not affect the expression of neighboring repeats, consistent with a single repeat unit being able to autonomously express histone genes, and by extension likely being able to nucleate a small HLB. The HLBs that form on each repeat unit then coalesce into a single HLB on an array, likely through liquid-liquid phase separation (Hur et al., 2019). Our edits to the revised version have tried to make these points more clear.

Reviewer #2 (Remarks to the Author):

The most prominent nuclear organelle in most eukaryotic cells is the nucleolus, which assemble at the locus of the ribosomal RNA genes. A less prominent, but probably ubiquitous nuclear organelle, is the histone locus body (HLB), which functions in the processing of histone pre-
mRNA. In this manuscript the authors examine the HLBs of Drosophila using a variety of genetic and cytological techniques.

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We thank the reviewer for his/her time and for appreciating the value of our study, particularly that the unique properties of the Drosophila histone gene repeats allow us to do these types of experiments, which are impossible in other organisms.
RE: Manuscript #E20-03-0176R  
TITLE: “Drosophila Histone Locus Body assembly and function involves multiple interactions”

Robert-
Bill-
I have looked over your revisions for this manuscript and find that they satisfactorily address the comments by referee 1. Referee 2 as supportive and had no requests. Thank you for choosing MBoC for publication of this very interesting study. 
Best wishes,
Tom

Monitoring Editor
Molecular Biology of the Cell

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Dear Dr. Duronio:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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