Dynamics of activating and repressive histone modifications in *Drosophila* neural stem cell lineages and brain tumors

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MS TITLE: Dynamics of activating and repressive histone modifications in *Drosophila* neural stem cell lineages and brain tumors

AUTHORS: Merve Deniz Abdusselamoglu, Lisa Landskron, Sarah K. Bowman, Elif Eroglu, Thomas Burkard, Robert E. Kingston and Jürgen A. Knoblich

I have now received all the referees' reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPressand click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, the referees express considerable interest in your work, but have some significant criticisms and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, I will be happy receive a revised version of the manuscript. Your revised paper will be re-reviewed by one or more of the original referees, and acceptance of your manuscript will depend on your addressing satisfactorily the reviewers' major concerns. Please also note that Development will normally permit only one round of major revision.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The authors purified distinct subtypes of Drosophila neural stem cells (NSCs) using FACS and subjected to ChIP-seq analyses for genome-wide distribution of H3K27me3 and H3K4me3. They

found that stem cell identity genes are silenced during differentiation of NSCs by loss of H3K4me3 but not through gain of H3K27me3. By contrast, PcG silencing is required for subtype specification of neuroblasts (NBs). This study provides important datasets for histone modifications in NSCs in vivo. I appreciate their great efforts to collect NSCs from larval brains. This study and the previous study of Marshall & Brand complement each other to reveal changes in the chromatin state during development and specification of NSCs.

Comments for the author

Followings are my comments that would facilitate to improve the manuscript.
1. It will be nice to add ChIP-seq data of H3K9me3. Although Marshall & Brand have reported the enrichment of HP1 in mira, CycE, stg and dpn in neurons, HP1 has been known to silence or activate genes in a context-dependent manner. Therefore, H3K9me3 data are important. It also significantly elevates the value as a resource.
2. Fig. 1A: NBI might be NBII in the NBII lineage panel. Fig. 1B: Where is a dashed line described in the Figure legend?
3. Fig. 2A. What is ACD?
4. Results, line 273. Fig. S2B should be Fig. S3B.
5. Results, line 276. Fig. S2C should be Fig. S3C.
6. Fig. 3D. What are the white and yellow arrowheads?
7. Fig. 3E. As general audience of Development is not so familiar with the fly NBs, it may be difficult to image the NB size from the figure. It will be nice to encircle a typical NBII with a dotted line in each close-up panel of Fig. 3E.
8. Legend to Fig. 4. (D) should be quantification of NBII numbers. (E) should be quantification of NBII diameter.
9. Results, lines 310-312. Something is wrong in the sentence. They have to rewrite it.

Reviewer 2

Advance summary and potential significance to field

This paper presents an analysis of selected epigenetic marks and transcription factors that specify cell identities of Drosophila neural stem cells (neuroblasts), their differentiated progeny, and that distinguish two major lineage subtypes (NB subtypes I and II). ChIP-seq is used to track genome-wide distributions of the histone marks H3-K4me3 and H3-K27me3. In addition, key transcription factors that operate in these lineages are tracked and functionally tested by knockdown. Finally, the role of Polycomb (PcG) repressors (in PRC1 and PRC2) in neuroblast maintenance, differentiation, and subtype specification are also assessed. The main findings extend and complement previous studies (Marshall and Brand 2017; Aughey et al. 2018) of chromatin factors and transcriptional regulators in Drosophila neuroblasts and their descendants. The findings here include identification of two transcription factors (Dll and eya) with roles in the NBII lineage as well as data that links PcG repressors to NBII subtype specification. In addition, the findings here are consistent with HP1-mediated (rather than PcG) silencing of stem cell renewal genes in neuroblasts, as previously described by others (Marshall and Brand 2017). Overall, I think this paper and dataset provide a useful addition to resources for deciphering epigenetic and regulatory circuitry in Drosophila neural stem cells and descendants. As elaborated below, I think the dataset (or at least the presentation) could be usefully addended with more information about specific PcG target genes that are functionally important in the NBII lineage.

Comments for the author

On pg. 13, lines 358-360, the authors speculate about PcG target genes with roles in NBIIs. Specifically, they propose that PcG repressors silence genes that induce apoptosis and promote differentiation. The final paragraph of the Discussion (lines 411-414) identifies specific PcG target genes in the NBII lineage (opa and ham), but I don't see either ChIP-seq or loss-of-function data to elaborate on how these might fit in to the circuitry. Where is the H3-K27me3 data on opa and ham that is referred to here? How can readers access it? I think the authors should add experiments to address this speculation or, minimally, a Figure panel that displays the K27me3 and K4me3 distributions on opa and ham in NBII cells.

Additional comments and questions:

1) In mammalian stem cell systems, “bivalent” chromatin domains (defined as genes that simultaneously contain abundant promoter-associated H3-K4me3 along with widespread H3-K27me3) have been noted as a common feature of differentiation genes. It’s been proposed that bivalent domains may help poise these target genes for rapid switching to active or fully silent modes. Inasmuch as this paper tracks these two chromatin marks, can the authors address whether bivalent domains are detected, and possibly relevant, in their dataset?

2) pg. 8, line 200. Please clarify: are the H3-K4me3 reads counted from +1 to +500 or from +500 to +1000 bp on target genes? If it’s the latter, why are promoter regions excluded?

Minor issues:

1) pg. 3, line 61. Clarify “…two differentiated neurons of glia.” Should “of” be “or”?

2) pg. 4, line 100: histone H2A, line 104: histone H3.

3) pg. 4, line 103: Not aware of a specific repressive histone acetylation event. Histone acetylation generally correlates with gene activity.

4) Fig. 1B, Legend – I don’t see a dashed line that separates optic lobe from central brain.

5) pg. 12, line 311. Fix duplicated text in sentence.

6) pg. 13, line 346-348. Fix sentence. As written, meaning is unclear.

First revision

Author response to reviewers' comments

We thank all reviewers and editors for their helpful comments and the opportunity to submit our revised manuscript. According to the reviewers’ suggestions, we included numerous modifications and additional analysis to strengthen our conclusions. Below is a detailed response to all their comments and the changes made in the manuscript are marked in yellow.

Reviewer 1:
Followings are my comments that would facilitate to improve the manuscript.
1. It will be nice to add ChIP-seq data of H3K9me3. Although Marshall & Brand have reported the enrichment of HP1 in mira, CycE, stg and dpn in neurons, HP1 has been known to silence or activate genes in a context-dependent manner. Therefore, H3K9me3 data are important. It also significantly elevates the value as a resource.

We thank the reviewer for the suggestion. We agree that the additional H3K9me3 ChIP-Seq data would be interesting. However, we believe the generation and associated analysis of additional ChIP-seq data would mean repeating much of the complete effort that went into assembling this paper. Our dataset provides a comprehensive resource to study chromatin dynamic during neurogenesis in vivo that condenses into a key message (genes are silenced during differentiation by loss of their activating marks and not via repressive histone modifications). However, we do agree with the reviewer that confirming the nature of HP1 activity will contribute to the manuscript.
Instead, therefore, we strengthen our dataset, by taking advantage of already published transcriptome data of neurons and NBIs and analyzed the relevant gene clusters (cluster 4) in terms of gene expression. We included this analysis in Figure 2. As evident from this data, we can conclude that the genes that belong to cluster 4 are downregulated in neurons compared to NBIs,

and our data shows that the levels of H3K27me3 levels are unchanged. These suggest that genes in this cluster are silenced via other mechanisms, probably via a repressive rather than activating HP1 mechanism.

2.Fig. 1A: NBI might be NBII in the NBII lineage panel. Fig. 1B: Where is a dashed line described in the Figure legend?
We changed the NBI to NBII. We also added the dashed lines to mark optic lobe.

3.Fig. 2A. What is ACD?
We have now written ACD as asymmetric cell division both in the figure and its legend.

4.Results, line 273. Fig. S2B should be Fig. S3B.
We changed the figure numbers.

5.Results, line 276. Fig. S2C should be Fig. S3C.
Changes were implemented.

6.Fig. 3D. What are the white and yellow arrowheads?
We added the explanation for yellow and white arrowheads in the figure legend.

7.Fig. 3E. As general audience of Development is not so familiar with the fly NBs, it may be difficult to image the NB size from the figure. It will be nice to encircle a typical NBII with a dotted line in each close-up panel of Fig. 3E.
We added dotted lines in close-up panels to mark NBs and make the size differences clearer.

8.Legend to Fig. 4. (D) should be quantification of NBII numbers. (E) should be quantification of NBII diameter.
We corrected this mistake.

9.Results, lines 310-312. Something is wrong in the sentence. They have to rewrite it.
We rewrote this sentence to: These results indicate that in addition to its function as anti-apoptotic in both type I and type II NB, PRC2 is required specifically in NBIIs to maintain self-renewal potential.

Reviewer 2:
On pg. 13, lines 358-360, the authors speculate about PcG target genes with roles in NBIIs. Specifically, they propose that PcG repressors silence genes that induce apoptosis and promote differentiation. The final paragraph of the Discussion (lines 411-414) identifies specific PcG target genes in the NBII lineage (opa and ham), but I don't see either ChIP-seq or loss-of-function data to elaborate on how these might fit in to the circuitry. Where is the H3-K27me3 data on opa and ham that is referred to here? How can readers access it? I think the authors should add experiments to address this speculation or, minimally, a Figure panel that displays the K27me3 and K4me3 distributions on opa and ham in NBII cells.
We thank the reviewer for this suggestion. We agree that unraveling the role of PcG targets in NBs would be the next step. Previously, it has been demonstrated that overexpression of both opa and ham, individually causes loss of NBIIs (Abdusselamoglu, Eroglu, Burkard, & Knoblich, 2019; Eroglu et al., 2014). Thus, we have speculated that the de-repression of these two genes might have a role in NBII-loss upon PcG-depletion. Since PcG-target genes involve more than only opa and ham in this case, together with the fact that generation of loss-of-function flies with PcG genes, p35, and the PcG-target genes is not feasible and beyond the scope of this manuscript. However, we have now added a supplementary figure showing the ChIP tracks for H3K4me3 and H3K27me3 for opa and ham.

Additional comments and questions:

1) In mammalian stem cell systems, "bivalent" chromatin domains (defined as genes that simultaneously contain abundant promoter-associated H3-K4me3 along with widespread H3-K27me3) have been noted as a common feature of differentiation genes. It's been proposed that

bivalent domains may help poise these target genes for rapid switching to active or fully silent modes. Inasmuch as this paper tracks these two chromatin marks, can the authors address whether bivalent domains are detected, and possibly relevant, in their dataset?
We agree with the reviewer that investigating bivalency will add to our characterization of histone modifications. We have included such analysis and discussion, which can be found in the main text at page 10.

2) pg. 8, line 200. Please clarify: are the H3-K4me3 reads counted from +1 to +500 or from +500 to +1000 bp on target genes? If it's the latter, why are promoter regions excluded?
We changed the sentence to explain this better. The reads were counted at the promoter region from +1 to +500bp.

Minor issues:

1) pg. 3, line 61. Clarify "…two differentiated neurons of glia." Should "of" be "or"?
We changed it to 'or'.

2) pg. 4, line 100: histone H2A, line 104: histone H3.
We corrected these mistakes.

3) pg. 4, line 103: Not aware of a specific repressive histone acetylation event. Histone acetylation generally correlates with gene activity.
We thank the reviewer to point out this mistake. We rewrote this sentence to make it clear. Now the sentence states that PcG proteins act as a repressor of histone methylation and acetylation.

4) Fig. 1B, Legend – I don't see a dashed line that separates optic lobe from central brain.
We included the dashed line now in the figure for better clarity.

5) pg. 12, line 311. Fix duplicated text in sentence.
We fixed the sentence.

6) pg. 13, line 346-348. Fix sentence. As written, meaning is unclear.
We changed the sentence.

REFERENCES
Abdusselamoglu, M. D., Eroglu, E., Burkard, T. R., & Knoblich, J. A. (2019). The transcription factor odd-paired regulates temporal identity in transit-amplifying neural progenitors via an incoherent feed-forward loop. eLife, 8, 450. http://doi.org/10.7554/eLife.46566
Eroglu, E., Burkard, T. R., Jiang, Y., Saini, N., Homem, C. C. F., Reichert, H., & Knoblich, J. A. (2014). SWI/SNF complex prevents lineage reversion and induces temporal patterning in neural stem cells. Cell, 156(6), 1259–1273. http://doi.org/10.1016/j.cell.2014.01.053

Second decision letter

MS ID#: DEVELOP/2019/183400

MS TITLE: Dynamics of activating and repressive histone modifications in *Drosophila* neural stem cell lineages and brain tumors

AUTHORS: Merve Deniz Abdusselamoglu, Lisa Landskron, Sarah K. Bowman, Elif Eroglu, Thomas Burkard, Robert E. Kingston, and Jürgen A. Knoblich
ARTICLE TYPE: Techniques and Resources Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

The authors purified distinct subtypes of Drosophila neural stem cells (NSCs) using FACS and subjected to ChIP-seq analyses for genome-wide distribution of H3K27me3 and H3K4me3. They found that stem cell identity genes are silenced during differentiation of NSCs by loss of H3K4me3 but not through gain of H3K27me3. By contrast, PcG silencing is required for subtype specification of neuroblasts (NBs). This study provides important datasets for histone modifications in NSCs in vivo. I appreciate their great efforts to collect NSCs from larval brains. This study and the previous study of Marshall & Brand complement each other to reveal changes in the chromatin state during development and specification of NSCs.

Comments for the author

The authors satisfactorily responded to my comments and revised the manuscript.

Reviewer 2

Advance summary and potential significance to field

As mentioned in my earlier review, this paper supplies a useful addition to resources for investigating regulatory circuitry in fly neural stem cells.

Comments for the author

This paper has been adequately revised to meet reviewers' requests. In particular, the authors have added Figure panels to display ChIP-seq results in the NBII lineage on the target genes ham and opa (Suppl. Fig. 4). Other minor changes have been made to the text as requested. With this updated version, I do not have any substantive requests for further modifications. One minor thing is that the following newly added sentence (lines 263-265) appears to be missing a word or two and should be fixed:

"Thus, our study in purified neuronal stem cells and together with previous reports (Gan et al., 2010; Marshall & Brand, 2017; Schuettengruber et al., 2009; Ye et al., 2016) adds to the growing that bivalent chromatin are absent in Drosophila."