Survival of Drosophila germline stem cells requires the chromatin binding protein Barrier-to-autointegration factor

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MS TITLE: Survival of Drosophila germline stem cells requires the chromatin binding protein Barrier-to-autointegration factor

AUTHORS: Tingting Duan, S. Cole Kitzman, and Pamela Geyer

I have now received reviews of your manuscript from 3 experts. The reviewers' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

As you will see, all 3 reviewers express strong interest in your findings. They offer numerous excellent suggestions for revising your manuscript before we can consider it for publication. All 3 reviewers suggest improvements to your figures and related text, including provision of numbers of samples analyzed, discussion of how variable the phenotypes are, the need for companion images, and the need to be consistent with genotype labeling. Reviewer 1 also notes that release of BAF from the nuclear lamina in germ cells has different consequences than loss of BAF, which challenges your interpretation. Reviewer 3 wonders if Otefin binds BAF, and notes that this small exploration of mechanism would enhance your manuscript. I would encourage that test if it is feasible.

I invite you to consider the reviewers' suggestions and submit a revised manuscript. Your revised manuscript will be re-reviewed, and acceptance will depend on your satisfactorily addressing the reviewers' concerns. Please note that Development normally permits only one round of 'major revision'.

In your revised manuscript, please clearly HIGHLIGHT all changes made in the revised version. You should avoid using 'Tracked Changes' in Word files as these are lost in PDF conversion. I also request 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 the reviewers' criticisms or suggestions, please explain why.

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Reviewer 1

Advance summary and potential significance to field

Correct assembly and function of the nuclear lamina is critical. In all Drosophila cell types the NL contains three different LemD proteins and a single BAF protein. BAF is particularly interesting because it is thought to link the NL to chromatin via its chromatin/DNA binding domain. But, despite extensive research, BAF assembly and function remains poorly defined. Prior studies suggest that BAF assembles into the NL via a physical interaction with all three LemD domain proteins. Functional studies show that BAF is essential; baf loss of function alleles cause early larval death. However, loss of single LemD proteins are not lethal and instead cause cell type specific phenotypes. This suggests a more complex paradigm in which the BAF-LemD proteins might display cell type specific dependencies. Indeed, this is what Geyer and colleagues have found. The data in this paper shows that (1) in germ cells and (2) 3rd instar imaginal wing discs that in the absence of the OTE LemD protein the majority of BAF is released from the NL. The authors then go on to focus on germ cells to show that the OTE mutant defects are similar to, but not identical to germ cell specific BAF loss. Remarkably, germ cells without BAF, like germ cells without OTE, can be rescued by atr or chk2 mutations. The key impact of this work, therefore, is the identification of a critical function for BAF in germ cell development and the discovery that OTE's primary role is to tether BAF to the NL lamina.

Comments for the author

In general, the presentation is clear and the data presented are of high quality and are interpreted appropriately. However, several issues were encountered regarding the presentation, as outlined below.

Major
(1) Fig. 1A: What is the n here? How variable is this phenotype? The magnification of the images needs to be the same across all the mutant phenotypes (to my eye, the cell sizes and the bar sizes are not the same). Furthermore, the ote mutant panel is highly pixilated, and the cells look larger than the cells in the other panel. The pictures in supplemental Fig. 1A are much more convincing. Consider swamping them out.

(2) Fig. 2B shows that ote mutant wing imaginal discs have increased cell death. The authors assume that it is due to the absence of BAF at the NL. If this is so, then one would expect to see a similar increase in cell death in discs from baf loss of function mutants (or in tissue-specific RNAi knock down discs). This experiment needs to be done and shown. These and other direct comparisons are needed to clarify whether loss of BAF gives the same phenotype as releasing BAF from the NL.

(3) Fig. S1C: What is the n here? How variable is the phenotype? As presented, these data are not nearly as convincing. I am also not sure what the point of adding this data to the manuscript is—salivary glands may be the same and may be different. Either way it does not add or detract from the story line. Perhaps the authors could simple remove this data.

(4) Fig. 3B: What is the n here? How variable is the phenotype? Are bocks and dMan mutants fertile?

(5) Fig. S2, the quantification of Fig. 4A should be moved to the main text.

(6) Fig. 4B. What is the n here? How variable is the phenotype?

(7) Fig. 4 I do not agree with the authors interpretation. In both ote mutants and baf RNAi mutants there is a clear distortion of the NL. But, in ote mutants there are about 1 HP1A blobs per cell. In baf RNAi mutants there are more HP1A blobs per cell. Which to me suggests that the release of BAF from the NL lamina in germ cells does not phenocopy a loss of BAF, which the opposite of what the authors state at the bottom of page 10. Later, the rescue experiments shown in Fig. 5 and 6 also indicate that the that the consequences of releasing BAF from the NL lamina is not the same as loss of BAF function. This tells me that the untethered BAF may be doing something in the cell… but

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what is it doing? One might be able to gleen a clue from the phenotype of an ote/ote; nos>baf RNAi double mutant germline.

(8) Fig. 5A needs companion pictures at the same magnification of nos>baf RNAi and wild-type.

(9) Fig. 6A needs companion pictures at the same magnification of nos>baf RNAi, ote mutants and wild-type.

Minor
(1) In the introduction, the authors should make it very clear when they are talking about mammalian cells or Drosophila cells.

(2) I could not identity statements regarding the evidence that BAF directly binds all three LemD proteins in Drosophila cells, and that all three LemD proteins are expressed together in all Drosophila cells. Interpretation of the data in this manuscript rests on the assumption that these two statements are true.

(3) Data validating the generation of an endogenous baf-GFP allele (fig. 1B, C and D) could be relegated to the supplemental data. On the other hand,Fig. S1B, the western blot showing that the amount of baf-GFP protein is about the same in WT as in ote mutant tissue could be moved to the main body of the manuscript.

(4) In most figures the germ cell specific driver is labeled nos and in the text and in Fig. S2 it is labeled nosgal4vp16. If it is the same driver used throughout then please be consistent in your labeling. Also, as gal4 driven RNAi experiments are extremely temperature sensitive, please state at which temperature the crosses were done at.

(5) Pairing of Fig. 3A with Fig. 4 seems more logical to me, as the phenotypes are matched. Consider showing individual ovarioles instead of the entire ovary, as was done in Figure S3C.

Reviewer 2

Advance summary and potential significance to field

This well-written manuscript reports important advances in understanding BAF (Barrier to integration factor), an essential protein that functions during S-phase, mitotic chromosome segregation and post-mitotic assembly of the nucleus, and is also an epigenetic regulator. These authors report the rigorous analysis of loss-of-function phenotypes for BAF alone, and in relation to loss of each of its conserved LEM-domain (‘LEM-D’) partners at the inner nuclear membrane, in the developing female germ cell niche in Drosophila. To overcome two long-standing challenges to BAF studies (antibodies routinely fail to detect endogenous BAF in cells; BAF has global and tissue-specific roles), they chromosomally inserted GFP at the N-terminus of BAF, and showed this GFP-BAF protein rescues all BAF-null phenotypes including fertility. They focused on the female germ cell niche, where one LEM-D protein (D-emerin/Otefin), is essential. Their genetic results are rigorous and definitive. They discovered that BAF association with the nuclear lamina depends on a single LEM-D protein, named D-emerin/Otefin; this finding is novel because Drosophila has two other LEM-D proteins, one of which is emerin-related (D-emerin2/Bocksbeutel). Their results, including rescue experiments and double-mutant studies of the checkpoint for nuclear lamina integrity, are excitingly the first evidence in any experimental system or tissue that BAF can depend on a single member of the LEM-D family. This work expands the genetic pathway for the nuclear lamina checkpoint, involving the ATR and Chk2 kinases, and has important implications for the mechanisms and consequences of BAF association with human emerin in the context of disease.

Comments for the author

Questions and clarifications:

1. Labeling and organization of Figure 3A:

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(a) Must label the four panels of Figure 3A individually (e.g., A,B,C,D) and refer to them individually in the results.
(b) The “nos>baf RNAi” experiment is never explained in results or legend for Figure 3, and the genetic background for the “nos>baf RNAi” panel is missing from Figure 3 (three different backgrounds; in which background was BAF downregulated?). Since the RNAi strategy is explained on page 10 for Figure 4, the manuscript would be improved by either:
(i) describing the RNAi strategy on page 9 to accompany re-labeled Figure 3D, or
(ii) [better?] move the nos>baf RNAi results to Figure 4.
(c) For Drosophila non-experts, the wildtype control (‘Fig 4A’) should be shown at the same magnification as other panels to facilitate comparison, and point to and explain significant features (e.g., 3 large round yellowish structures in ‘nos>baf RNAi’ panel?).

2. Labeling and organization of Figure 4:
(a) Must label the four upper panels of Figure 4A individually (e.g., A,B,C,D) and refer to them individually in the results (e.g., Figure 4D, upper/lower).
(b) For Drosophila non-experts like me, it will greatly improve the manuscript to point to and explain features of interest in the higher-mag wildtype image (‘Fig 4A, lower panel’). E.g., Vasa (red) localizes in the nucleus of germ cells (which lack heterochromatin?), and the Hp1-positive (green) structures are heterochromatin in which kind of cell?
(c) Add the genetic background for the “nos>baf RNAi” experiment to Figure 4.

3. Incomplete description of the ‘heterochromatin aggregation’ phenotype in Figure 4. (Appreciating the difficulty of imaging these cells): there are ~5-10 green objects in the wildtype image (Fig 4A, lower panel); this normal phenotype is called ‘dispersed’ – do these green signals correspond to silent chromatin in 5-10 nuclei?

The ote-/- animals have one huge Hp1-positive feature (‘aggregated’ phenotype) with a brightness similar to the summation of all 5-10 individual signals in wildtype. This phenotype looks like an extreme case of the aneuploidy that results from defective chromosome segregation in C. elegans that lack BAF, or two LEM-D proteins. Can the authors comment, for example on discussion page 15? E.g., could this ‘aggregate’ be a single (~5x aneuploid) nucleus, or 5-10 nuclei clumped together? The term ‘aggregated’ is useful for scoring images, but these beautiful mutants may have more to reveal. Is this aneuploidy? incomplete nuclear envelope formation? S-phase dysregulation? defective cytokinesis or something else new?

4. Page 15,“epigenetic regulator (Montes de Oca et al, 2011).” Is it potentially relevant that BAF and transcription factor GCL (germ cell-less) compete for binding to emerin in vitro (Gjerstorff MF et al., 2012; Holaska JM et al., 2003)?

5. Clarify the model shown in Figure 6C. Arrows usually indicate positive regulation. Since loss of BAF activates Chk2, and loss of d-emerin activates ATR (for example), change these arrows to inhibition (lines ending in bar)?

Minor revisions:

Page 4— Include the human BAF gene name (BANF1) to avoid confusion with Brahma-Associated Factors.

Page 4, para-2, line 6: change to “for chromatin attachment to the NL during post-mitotic nuclear assembly”.

Page 4, para-2, line 7: rephrase to avoid the erroneous impression that BAF only interacts with A-type lamins during post-mitotic nuclear assembly (C. elegans has one lamin, ‘B-type’).

Page 9: “contributes” should be “contributions”

Page 13, middle-para, sentence that starts “BAF dispersal..”: change to “Loss of d-emerin/otefin is sufficient to disperse BAF in cells that still express A- and B-type lamins...”

Page 13, bottom-para; fix the grammar in “Pairwise alignments..” sentence.

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Page 13, 2nd line from bottom: Change to “Self-association of emerin has been found...”

Page 14, line 5: for “nutrient availability”, could also cite the 2014 paper by Bar,DZ et al.

Page 14, middle paragraph: “Cell cycle regulation” implies a ‘regulatory’ role for BAF. Would it be more accurate to say, e.g., “essential BAF function relates to mitosis, including chromosome segregation (Samwer et al., 2017) and nuclear lamina assembly.”?

Page 15: delete the sentence “Indeed, loss of BAF diminishes levels of NL D-emerin/Otefin (data not shown)...”. Unnecessary and detracts by raising unanswered questions (detection/epitope accessibility).

Page 25: Spell out DCP-1 in the figure legend.

Reviewer 3

Advance summary and potential significance to field

This manuscript explores the relationship between the nuclear lamina component Otefin/Emerin and BAF in the regulation of germline stem cell maintenance in the Drosophila ovary. In previous work BAF has been implicated in nuclear assembly, chromatin organization, gene expression and gamete development.
Notably, the composition of the nuclear lamina is cell type specific and thus likely contributes to the integration of signals from various developmental signaling pathways. For this reason, the manuscript will appeal to a broad audience with interests in the nuclear lamina, stem cells and germline development.
Specifically, the authors determine that the Drosophila Emerin homolog Otefin functions with BAF in the maintenance of the adult germline stem cell population. This manuscript contributes to our understanding of how proteins in the nuclear lamina (including LEM-D proteins such as Otefin) can make tissue specific contributions to cell survival and cell fate.

Comments for the author

Major Comments

1. On page 8 the authors conclude that Loss of NL BAF increases cell death in larval tissues. This conclusion is based on two observations (1) In otefin mutants BAF is no longer concentrated on the nuclear lamina. (2) otefin mutants have increased cell death in developing imaginal discs. The authors never directly demonstrate that the loss of BAF from the NL is the cause of the observed cell death in otefin mutants. It is entirely possible that the cell death is related to another function of otefin. Therefore this idea should be presented as a hypothesis and not a conclusion.

2. The readers would benefit from a more complete description of the data presented in figure 4 in the figure legend. Also, the N for this experiment and how the nuclei were scored is not apparent. Would it be possible to include the quantification presented in Sup Figure 2 in Figure 4?

3. On page 10 the authors state “These experiments revealed that loss of BAF blocks germ cell differentiation and promotes GSC loss.” The authors need to link this statement to a figure or reference.
Currently, there is no data to support this statement.

4. Does Otefin bind BAF? A small exploration of mechanism would certainly enhance this manuscript.

5. The authors never formally documented the loss of germline stem cells in BAF RNAi depletions.

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Minor Comments

1. In the second to the last paragraph on page 9 the last sentence does not make sense. I suspect the word otefin is missing.

2. What is the N for the data presented in Figure 3?

3. In the results section at the top of page 10 the authors suggest that loss of NL BAF phenocopies a BAF over-expression phenotype. This one sentence of speculation is a bit confusing at this point in the manuscript. The authors either need to expand on this idea or save it for the discussion.

4. On page 11, in the concluding sentence of the second paragraph, the authors state “Based on these data (Figure 5) we conclude that BAF contributes to the activation of the NL checkpoint but has additional roles required for GSC survival. “This is speculation and should be presented as such. I agree that otefin mutants phenocopy BAF depletions with respect to the NL phenotype. However, whether this triggers an NL specific checkpoint is untested.

5. The authors should present a more complete picture about the known role of BAF in the female germline of Drosophila.

First revision

Author response to reviewers' comments

Reviewer #1:
Major Comments:
1. Images in Fig. 2A need to be the same magnification, pixilation of the d-emerin/otefin mutant panel needs to be corrected, and the # of nuclei studied needs to be indicated, along with a statement about variability of phenotype.

Response: We have reorganized Fig. 2, advancing part A into Fig. 1 (now Fig. 1C). We have corrected the quality of the images and magnification as requested. The number of nuclei that were studied is noted in the figure legend. In the text we state:

“Staining for GFP-BAF revealed a consistent nuclear phenotype within a field of imaginal disc cells, wherein GFP-BAF is enriched at the nuclear periphery and shows a strong overlap with lamins (Fig. 1C, Fig. S2A)”.

2. Fig. 2B showed that loss of D-emerin/Otefin increased cell death. However, levels of cell death in baf mutant discs were not presented, an experiment that needs to be done. These studies are needed to clarify if loss of BAF gives the same phenotype as loss of NL BAF.

Response: Complete loss of BAF results in the absence of imaginal discs (Furukawa et al., 2003). For this reason, we generated baf∆24/baf1 mutant animals because baf∆24 allele is hypomorphic, allowing recovery of baf mutant animals with imaginal discs. Notably, no baf∆24/baf1 adults emerge. The baf∆24/baf1 mutant discs showed higher levels of cleaved DCP-1 staining than the ote mutant discs. Whereas these data show that NL loss of BAF is less severe than total compromised BAF function, the increased staining of ote mutants is consistent with the proposal that NL BAF has some function. This section now reads:

“BAF is an apoptotic mediator (Furukawa et al., 2007). Yet, loss of D-emerin/Otefin has no effect on organism viability (Barton et al., 2014), indicating that loss of BAF within the NL might not be sufficient for induction of apoptosis. To test this possibility, we stained wild type, d-emerin/otefin mutant and baf mutant imaginal discs with antibodies against a marker of apoptosis, the Drosophila effector cleaved Death Caspase 1 (DCP-1; Fig. 2A). We chose baf1/bafD24 animals for these studies, because baf∆24 is a hypomorphic allele that allows enough BAF function to recover larvae

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with imaginal discs, but not viable adults. We found that d-emerin/otefin and baf1/bafD24 mutant discs showed DCP-1 staining, with higher levels of staining in baf1/bafD24 mutant discs (Fig. 2A). Based on these data, we suggest that delocalization of NL BAF might partially compromise BAF function, leading to increased apoptosis."

3. In Fig. S1C, the n needs to be listed, along with a statement of variability of the phenotype. The salivary gland data are more difficult to interpret, and the results of these analyses do not add or detract from the story line, so these data could be removed.

Response: We have re-organized the figures, so S1C is now S2B. In the figure legend, we now state that the nucleus shown is representative and that at least five nuclei were scanned, with similar results obtained. We wish to keep the salivary gland data in the manuscript, as these data demonstrate that both D-emerin/Otefin and D-emerin2/ Bocksbeutel contribute to nuclear localization of BAF.

4. Fig. 3B, the n needs to be listed, along with a statement of the variability of the phenotype.

Response: This has been done, as described in the response to comment #3.

5. Fig. S2, the quantification of Fig. 4A should be moved to the main text.

Response: This has been done. These data appear in Fig. 5 (5B).

6. Fig. 4B, the n and variability of the phenotype needs to be addressed.

Response: We have used the measurement of the nuclear lamina thickness as a method to define and quantify the effects of loss of D-emerin/Otefin and BAF on the nuclear phenotype. These data are shown in Fig. 5B, with the number of nuclei studied shown over the box plot.

7. The interpretation of data in Fig. 4 was questioned. It was noted that although the NL phenotype of nos>baf RNAi and d-emerin/otefin mutants is similar, nuclear localization of HP1a differs. This reviewer notes that these observations suggest that release of BAF from the NL in germ cells differs from complete loss of BAF. Can more information be gleaned from ote-/-; nos>baf RNAi double mutants?

Response: We completely agree that while effects of loss of NL BAF share similarities with those resulting from BAF knockdown, there are differences. Indeed, our data indicate that "untethered" BAF contributes to the HP1a aggregation that is seen in d-emerin/otefin mutant GSCs (Fig. 5). We have tried to examine the GSC phenotype in ote-/-; nos>baf RNAi double mutants, but unfortunately, these ovaries completely lack germ cells.

We apologize that that we failed to convey the distinction between loss of NL BAF and those resulting from BAF knock down. We have re-written the conclusion on page 10. It now reads:

"Next, we investigated nuclear phenotypes in baf RNAi GSCs. Ovaries were dissected from newly eclosed nos>baf RNAi females and co-stained with Vasa antibodies to detect germ cells and either antibodies against Lamin to examine NL phenotypes or Heterochromatin Protein 1a (HP1a) to examine the distribution of heterochromatin (Fig. 5A, C; S4). These analyses revealed that BAF knockdown causes a thickened and irregular NL structure, without heterochromatin aggregation. In comparison, d-emerin/otefin mutant nuclei display a thickened and irregular NL with heterochromatin aggregation (Fig. 5; S4). Based on these data, we conclude that BAF knockdown shares some, but not all, defects associated with loss of D-emerin/ Otefin."

In addition, in the discussion, we discuss our thoughts on effects of loss of BAF at the NL (see section Phenotypes associated with loss of NL BAF differ from those of complete BAF loss) relative to those resulting from complete loss of BAF (see section Survival of adult stem cells depends upon BAF).

8/ 9. Fig. 5A and 6A need companion pictures of the same magnification of nos>baf RNAi, ote-/- and wild type.

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Response: This has been done. These figures are now Fig. 6A and 7A.

Minor Comments:
1. In the introduction, clarify whether talking about mammalian cells or Drosophila.

Response: Interactions between the LEM-D and BAF has been established for human, flies and worm proteins. Even so, much of the in vitro work has used human BAF, whereas in vivo work has been completed in human cells, flies and worms. Our references include all organisms and refer to metazoan cells to indicate the generality of the statements. The introduction second paragraph now reads:

"The defining feature of this conserved family is the LEM Domain (LEM-D), an ~40 amino acid domain that directly interacts with the metazoan chromatin binding protein Barrier-to-autointegration factor [BAF, sometimes referred to as BANF1; (Lin et al., 2000, Zheng et al., 2000, Cai et al., 2001, Montes de Oca et al., 2005, Pinto et al., 2008, Liu et al., 2003)]. Purified human BAF directly binds double-stranded DNA, the A-type lamin, and histones in vitro (Montes de Oca et al., 2005, Zheng et al., 2000, Umland et al., 2000, Samson et al., 2018, Brachner and Foisner, 2011, Lancaster et al., 2007, Lee et al., 2001), suggesting that BAF also promotes chromatin-NL connections using non-LEM-D dependent mechanisms. In dividing metazoan cells, regulated formation of complexes between LEM-D proteins, BAF and lamin controls mitotic spindle assembly and positioning, as well as the reformation of the nucleus (Margalit et al., 2005, Samwer et al., 2017, Qi et al., 2015, Mehsen et al., 2018). In non-dividing metazoan cells, LEM-D proteins and BAF cooperate to tether the genome to the nuclear periphery and form repressed chromatin (Gonzalez-Aguilera et al., 2014, Jamin and Wiebe, 2015)."

2. A statement regarding BAF directly binding to the Drosophila LEM-D proteins was hard to find, as well as information that all three LEM-D proteins are expressed together in all Drosophila cells.

Response: This information is now included in the introduction. The text in the third paragraph now reads:

"Drosophila encodes three NL LEM-D proteins that bind BAF (Pinto et al., 2008), including two emerin orthologues (D-emerin/Otefin and D-emerin2/ Bocksbeutel) and dMAN1."

3. Data validating the generation of an endogenous baf-GFP allele can be relegated to the supplemental data.

Response: This has been done. These data are in Fig. S1.

4. Labeling of the gal 4 driver is inconsistent. Also, what was the temperature of the RNAi experiments.

Response: In the germline knockdown experiments, we used the same driver, a nanos promoter driven GAL4-VP16 activator. We have corrected the figures for uniform labeling. They now read: nos> bafRNAi. In addition, we have added experimental detail in the results section. It now reads:

"In these studies, animals carrying a UASp transgene encoding a baf hairpin RNA were crossed with animals carrying the germline specific nanos (nos)>baf RNAi. All RNAi experiments were done at 25oC."

5. Figure 3A and Figure 4 should be paired.

Response: We appreciated this suggestion but felt that this pairing would impact the quality of the images, as we would have to reduce size. However, we did extensively reorganize figures for clarity.

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Reviewer #2:
Questions and clarifications:
1. Labeling and organization of Figure 3A needs attention. First, the panels need to be individually labeled. Second, the nos>baf RNAi experiment is not explained in this section and is only explained in reference to Figure 4. Third, pictures of ovaries should be shown at the same magnification.

Response: We appreciate these comments and have extensively reorganized our figures for clarification. First, we have simplified the old Figure 3, moving data into new Figures 3, 4. In new Figure 3, we include a cartoon of the ovary and germaria structure for reference for non-Drosophila experts. In new Figure 4, we show enlarged pictures of ovary phenotypes of wild type (baf+/+, ote+/+), d-emerin/otefin mutant (baf+/+, ote-/-) and d-emerin/otefin mutant with reduced BAF levels (baf+/-, ote-/-) and baf knockdown (nos>baf RNAi). Unfortunately, due to the size of the wild type ovary, we cannot show the wild type ovary at the same magnification. In these new images, we have marked some of the empty germaria with an * to show the complexity in the phenotype. Second, the reorganization of the data provided us with the opportunity to introduce experimental details of the baf RNAi study earlier.

2. Labeling and organization of Figure 4 needs attention. Further, non-Drosophila experts would benefit from a better description of the ovary and cell types under study. The genetic background for the nos>baf RNAi experiment needs to be added.

Response: The new Figure 4 has been simplified, and the panels are labeled with the appropriate genotype. Also, we established a new Figure 5.

This reviewer's questions concerning genetic background are unclear to us. We note the genetic background is nos>baf RNAi. We believe this confusion might have arisen because the nos>baf RNAi experiments were described later than the appearance of the figure.

3. There is incomplete description of the heterochromatin aggregation phenotype. What are these foci and to they represent silent chromatin in 5-10 nuclei? Further, are changes in HP1a staining due to aneuploidy?

Response: In the revised manuscript, we clarify these studies. We have included a section in the material and methods on how HP1a foci were counted. Further, we clarify that the images shown in new Figure 5 represent the HP1a distribution in a single nucleus. We expanded the explanation of our reasoning behind comparing the HP1a distribution in d-emerin/otefin mutants (ote-/-) versus these mutants with reduced levels of BAF (baf+/-, ote-/-). We believe that our observation of reversal of the aggregation phenotype in the baf heterozygotes is more consistent with increases remaining levels of nucleoplasmic BAF than with aneuploidy. This section now reads:

"To gain a better understanding of the differences in heterochromatin distribution between d-emerin/otefin mutant and baf RNAi ovaries, we studied GSC phenotypes in ovaries dissected from d-emerin/otefin mutant females with reduced BAF levels (baf+/-, ote-/-). Strikingly, in these ovaries, heterochromatin became dispersed (Fig. 5C, D; S4), indicating that BAF contributed to the HP1a coalescence in d-emerin/otefin mutant GSCs. We propose that in the absence of D-emerin/Otefin, untethered BAF increases nucleoplasmic pools, leading to increased chromatin association. We suggest that this loss of NL BAF is similar to the previously reported BAF over-expression phenotype (Montes de Oca et al., 2011)."

4. Page 15, is BAF and Germ cell-less competition with emerin relevant to the epigenetic regulation?

Response: This reviewer makes a very interesting connection. However, as we have no information concerning interactions between D-emerin/ Otefin and Germ cell-less, we did not include this as possibility.

5. Clarify the model shown in Figure 6C.

Response: We appreciate this reviewer's careful observation and have made the appropriate modifications. The model is now shown in Figure 7C.

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Minor revisions:
1. page 4- include the human gene name BANF1.

Response: We appreciate the confusing nomenclature. However, in our introduction we never refer to the human gene, which is called BANF1. We only refer to the protein, which in the literature and in the human gene database GeneCards is called BAF. Even so, to reflect the complication in gene naming, we included the following:

"The defining feature of this family is the LEM Domain (LEM-D), an ~40 amino acid domain that directly interacts with the metazoan chromatin binding protein Barrier-to-autointegration factor [BAF, sometimes referred to as BANF1; (Lin et al., 2000, Zheng et al., 2000, Cai et al., 2001, Montes de Oca et al., 2005, Pinto et al., 2008, Liu et al., 2003)]."

2. page 4- change to "for chromatin attachment to the NL during post-mitotic nuclear assembly".

Response: This change has been made.

3. page 4- rephrase the erroneous impression that BAF only interacts with the A-type lamins during post-mitotic nuclear assembly (C. elegans has one lamin, B-type)

Response: In the revised manuscript, we have clarified this section and indicated that the discussion refers to in vitro properties of purified human proteins, which show a bias towards interaction with the A-type lamin (Samson et al., 2018).

4. page 9: contributes should be contributions

Response: Done

5. page 13: "BAF dispersal…." Change to "Loss of D-emerin/Otefin is sufficient to disperse BAF in cells that express the A- and B-type lamins…."

Response: Done

6. page 13: Change "Self-association of LEM-D proteins has been found" to "Self-association of emerin has been found"

Response: Done

7. Page 14: cite paper by Bar, DZ et al.

Response: Done

8. page 14: Cell cycle regulation implies a regulatory role—would it be more accurate to say that "the essential BAF function relates to mitosis".

Response: Changed as suggested.

9. page 15: delete the sentence "Indeed, loss of BAF diminishes….:

Response: Done

10. Spell out DCP-1 in the figure legends.

Response: Done

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Reviewer #3:
Major comments

1. On page 8, the authors state that "Loss of NL BAF increases cell death in larval tissues", but they never directly demonstrate that it is the loss of NL BAF. Therefore, this idea should be presented as a hypothesis not a conclusion.

Response: We have changed the heading to more accurately reflect the data. The heading now reads:

"Loss of D-emerin/Otefin increases cell death in larval tissues"

2. The readers would benefit from a more complete description of the data in Figure 4, including the number studied. It is suggested that supplemental data be moved together with data in this figure.

Response: This reviewer is referring to what is now Figure 5. As noted in comments to reviewer #1 (comments 5-6) and reviewer #2 (comment 1), we have added a schematic of the Drosophila ovary and germarium for clarification of identification of GSCs (new Fig. 3A). In addition, we changed Figure 5 so that it only shows individual GSC nuclei, with the corresponding germarium shown in Fig. S4. We have moved the quantification of Fig. S2 into the main figure, now Fig. 5D. We have also included expanded the figure legend. The number of nuclei studied in these experiments is noted in as the numbers over the box plot for each genotype (Fig. 5B, D).

3. On page 10, the authors state "These experiments revealed that loss of BAF blocks germ cell differentiation and promoters GSC loss". The authors need to link this statement to a figure.

Response: We have rewritten this section to clarify why we made this conclusion and to include the figure reference. This now reads:

"These experiments revealed that similar to loss of D-emerin/Otefin, loss of BAF generates a complex phenotype, wherein many germaria lack germ cells, whereas other germaria have germ cells and even maturing stages of oogenesis (Fig. 4)."

4. Does Otefin bind BAF?

Response: The short answer is yes. We have clarified this point in the revised manuscript. Please see our response to reviewer #1, minor comment #2.

5. The authors never formally documented the loss of germline stem cells in BAF RNAi depletions.

Response: The loss of GSCs in BAF RNAi depletions is shown in two Figures. First, the representative ovary shown in Fig. 4, nos>baf RNAi has many germaria that lack cells stained for Vasa, germ cells. This demonstrates a loss of GSCs. In this revised figure, we now show some of the empty germaria with *. Second, we have quantified the % of germ cell containing germaria for nos>baf RNAi and for atr, nos>bafRNAi and chk2, nos>baf RNAi and these data are shown together in Fig. 6C. Notably, a median of ~60% are empty or have GSC loss. Only the chk2, nos>baf RNAi double mutant shows partial rescue of GSC loss, decreasing empty germaria to ~40%.

Minor comments:
1. The word Otefin is missing on page 9.

Response: This has been corrected.

2. What is the N for data in Figure 3?

Response: We have included n for data in the figure legends. In Figure 3B, the line scans were completed on at least 5 nuclei. For determination of the ovary and GSC nuclear phenotypes, experiments were performed using at two to three biological replicates involving at least five pairs of ovaries per experiments. Quantification of phenotypes was completed on the indicated number

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of germaria from at least five different ovaries per replica. We expanded the materials and methods to include this information.

We have added this information in supplementary information. In that document, we included a marked up word document showing differences between the revised manuscript and the former submitted manuscript.

Second decision letter

MS ID#: DEVELOP/2019/186171

MS TITLE: Survival of Drosophila germline stem cells requires the chromatin binding protein Barrier-to-autointegration factor

AUTHORS: Tingting Duan, S. Cole Kitzman, and Pamela Geyer
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks. The reviewers' comments are appended below. Please work with the journal to remove the duplicate reference noted by Reviewer 2.

Reviewer 1

Advance summary and potential significance to field

The authors have satisfactorily responded to all my questions and made the necessary changes to the manuscript. I recommend proceeding with publication.

Comments for the author

I recommend proceeding with publication.

Reviewer 2

Advance summary and potential significance to field

This revised manuscript is beautifully clear with convincing results, and fully satisfies all previous concerns. This study provides major insight into the individual roles of BAF and nuclear membrane protein emerin, and their functional interdependence in germline stem cells. This incisive dissection of two complicated and functionally-nuanced proteins has uncovered fundamental roles for each. This is particularly exciting for emerin as a functionally-redundant LEM-domain protein, and a major advance in understanding nuclear structural dynamics in biology and laminopathy disease.

Comments for the author

Minor correction: The 'Barton et al. 2018' citation is duplicated in references.

Reviewer 3

Advance summary and potential significance to field

This manuscript explores the relationship between the nuclear lamina component Otefin/Emerin and BAF, in the regulation of germline stem cell maintenance in the Drosophila ovary. In previous

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work BAF has been implicated in nuclear assembly, chromatin organization, gene expression and gamete development. Notably, the composition of the nuclear lamina is cell type specific and thus likely contributes to the integration of signals from various developmental signaling pathways. For this reason, the manuscript will appeal to a broad audience with interests in the nuclear lamina, stem cells and germline development. Specifically, the authors determine that the Drosophila Emerin homolog Otefin functions with BAF in the maintenance of the adult germline stem cell population.
This manuscript contributes to our understanding of how proteins in the nuclear lamina (including LEM-D proteins such as Otefin) can make tissue specific contributions to cell survival and cell fate.

Comments for the author

The authors have addressed all of our concerns.

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