An autoregulatory switch in sex-specific *phf7* transcription causes loss of sexual identity and tumors in the *Drosophila* female germline

Anne Smolko, Laura Shapiro-Kulnane and Helen Salz
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MS TITLE: An autoregulatory switch in sex-specific phf7 transcription causes loss of sexual identity and tumors in the Drosophila female germline

AUTHORS: Anne Smolko, Laura Shapiro-Kulnane, and Helen Salz

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 BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so. You will note that Reviewer 2 is particularly critical and would like to see a number of new data points to support your claims. Having read the paper and considered the reportsof the other reviewers, it is my view that the concerns of Reviewer 2 are not fatal flaws but rather are points that, if addressed, could strengthen the MS by adding clarification of some of your observations, including especially the transcriptional status of the genes that appear to be induced by phf7, in the wild type female germ line.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Reviewer 1

Advance summary and potential significance to field

Establishing sexual identity of germ cells is crucial for all sexual reproduction. In Drosophila, expression of PHF7 in testis promotes male identity. Conversely, the expression of phf7 is repressed in the females by a critical regulator of sexual identity, Sex lethal (Sxl). Loss of sxl results in formation of tumors and upregulation of phf7. Ectopic expression of PHF7 independent of sxl downregulation also causes germline death and formation of tumors. This hints at the possibility that PHF7 acts downstream of Sxl to masculinize the female germline.

In this study Smolko et al. have shown data and make claims that:

1. Ectopic expression of Phf7 in the female germ line causes a positive feedback loop wherein PHF7 overcomes its own transcriptional silencing by promoter switching.

2. Zinc finger domain of Phf7 is required for promoting the positive feedback loop.

3. PHF7 causes a "transcriptional reprogramming", allowing expression of "male genes" in the female germline with the caveat that the majority of the male genes expressed in the female germ cells when PHF7 is ectopically expressed are not controlled by PHF7 in the male germ cell.

Conceptual advance: As Phf7 is a chromatin reader that recruits transcriptional machinery, their model, if correct, suggests that different male specific genes are poised for expression in different tissues. Thus presence or absence of a male specific chromatin reader can license sex specific genes in different tissues.

Comments for the author

Major Problems with data: While the authors have shown evidence that there is a positive feedback loop for phf7 – their other two claims are not substantiated as outlined below:

Suggestions for authors:

1. My biggest concern is the RNA seq presented in this manuscript. I worry about comparing a phenotype that results in 70% germ cell death or 30% tumorous growth to WT ovaries. The overexpression of Phf7 results in accumulation of undifferentiated stages and this is compared to WT which enriches for late stage egg chambers. How can the authors control for this?

If they want to make this comparison, they should perform principle component analysis for Phf7 OE data set with data sets derived from critical stages of germ line differentiation such as GSCs and CBs that are already available. Then, they can determine which stage is the best to compare against.

2. They authors make a strong claim that a subset of genes upregulated upon the over expression of PHF7 are expressed only in the testis. To support that claim, they need to show that these genes are not expressed in the undifferentiated stages of the female germline. In addition, the authors should validate these targets either by using in-situs, antibodies or reporter lines where available. If these genes are already expressed in the female germline, then their claim falls apart.

The Salz lab has shown that bam mutants have been shown to express testis genes. To get around not using mutations, they could compare their data set to RNA seq data generated from virgin flies which enriches for stages present in the germaria.

3. In Figure 1, the authors find that by over expressing both WT and protein lacking PHD domain that only overexpression of full-length protein causes germline tumors. They interpret this data as evidence that PHD domain is important for function. However, to make this claim, the authors need to show that delta PHD PFH7 is in fact expressed at the protein level. I understand that an antibody is not available for PHF7. However making a tagged version of this protein and showing that it is expressed is critical to making this claim.

In lines 150-162, the authors suggest that more copies of PHF7 result in a more detrimental phenotype.
However, more copies do not mean that there is more protein. The authors need to show that along with increased PHF7 copies there is a correlated increase in the protein levels.
Minor comments:

- In Figure 1B, it seems as though the magnification for each image is different. A scale bar should be included for each image.
- In line 111 the authors suggest that the germaria fail to make an oocyte. The authors should stain for oocyte markers to show that the oocyte is not specified or otherwise change their wording.
- In line 114 the authors state that the flies lacking PHD domain were fertile. To truly state this, the authors should perform fertility tests on ectopically expressed flies and the flies lacking PHD in PHF7.
- In Figure 2B the authors show representative images of how ectopic PHF7 expression stimulates expression of reporter vectors. However, the images in 2B and 2C are different sizes.
- In Figure 2B, the authors' experimental image seems to lack any background green. If an antibody was used, one would expect some kind of background fluorescence. Please make sure all the channels were included.
- There is no scale bar for FPKM values in 4D.

Reviewer 2

Advance summary and potential significance to field

Summary and Critique Despite common developmental origins, oocytes and sperm undergo unique developmental programs leading to differentiation. Sexual identity in germ cells and surrounding somatic cells is a key determining factor in oocyte development. Although initiation of oocyte-specific transcriptional programs and coordinated repression of sperm-specific programs must occur to promote the oocyte program, the molecular mechanisms remain unclear.

In this study, Salz and colleagues build from previous observations that loss of the sexually-dimorphic RNA binding protein Sxl results in germ cell tumors that express Phf7, a male-specific transcription factor. Ectopic expression of Phf7 in female germ cells blocks oocyte differentiation, resulting in loss of germline stem cells and tumorous germaria. Salz and colleagues seek to determine the extent to which Phf7 can drive the male transcriptional program in female germ cells. Using novel genetic tools (including a GFP knock-in to the Phf7 open reading frame), functional studies of the Phf7 protein domains and transcriptomic analysis, the authors present evidence in support of a model by which Phf7 in female germ cells ectopically promotes a male transcriptional program.

Overall, this well-constructed study adds exciting new molecular understanding of maintenance of sexual identity in germ cells and its links to tumorigenesis. The study advances the field by identifying genes transcriptionally regulated downstream of Phf7. Experiments are well-controlled and introduce novel reagents that will be beneficial for the field.
This short report is appropriate for Development and its readers and is well-written. My specific comments/suggestions for improving the manuscript are detailed below (listed in the order in which they appear in the manuscript).

Comments for the author

Major points
1. Results, Figure 1 (and accompanying text, lines 114-120). The authors conclude that deletion of the PHD domain renders ectopic Phf7 inactive.
However, this conclusion hinges on whether the UASz-phf7[deltaPHD] actually makes a stable protein. As the authors mention, the best analysis here would be to use Phf7 antisera to make sure that a protein is actually produced. (Phf7 antisera was published in Yang et al. Van Doren 2017, Genetics 206:1939…but perhaps these antibodies were generated against the PHD domain or don't

recognize the ectopic ovarian protein? The authors should clarify their statement here.) If anti-Phf7 antisera are not available, another possibility might be to test whether the UASz-phf7[deltaPHD] causes any phenotypes in male germ cells that recapitulate phf7 mutants or fail to rescue the mutants.

2. Results, lines 150-162. I'm confused by the authors' conclusions in this section. I agree that there is an interesting observation here: the three different experimental paradigms (phf7[deltaORF]::GFP;nos>UASz-phf7 vs. nos>UASz-phf7 in a wild-type background vs. nos>phf7[EY]; HA-phf7) yield different penetrance of the tumor phenotype. But they also give different penetrance of the agametic phenotype, suggesting stronger ectopic activity of Phf7 (rather than weaker activity, as the authors conclude). Indeed, in the truly ectopic paradigm (phf7[deltaORF]::GFP;nos>UASz-phf7, driven off the very strong nos-Gal4::VP16 but in which all regulatory information for phf7 is intact), the vast majority of the germaria (according to Fig. 1C) are agametic…wouldn't lack of germ cells (ostensibly caused by loss of GSCs) trump the tumor phenotype? I am wondering (this is pure speculation) if somehow the regulatory information is different between the three experimental paradigms perhaps causing the phf7[deltaORF]::GFP;nos>UASz-phf7 to be more strongly expressed in larval/pupal germ cells prior to adulthood (so that GSCs aren't properly established) or in GSCs (so GSCs are lost more rapidly). Alternatively, there could be weaker expression from the nos>phf7[EY]; HA-phf7, which might also explain these effects.

In any case, the authors' statement that "We therefore conclude that the level of PHF7 protein dictates the phenotypic outcome, with the highest levels required for tumor initiation" could be experimentally tested with Western blots if suitable antibodies can be identified (see comment 1 above). If suitable reagents cannot be procured, I suggest that the authors clarify their conclusions in this section with a broader discussion.

Minor Points:
1. Figure 1: Statistics are missing for panel C
2. Figure 2, panels B-C aren't the same size (and are relatively small overall for the figure).
3. Figure S1, panels in C are too small relative to the rest of the figure.

Reviewer 3

Advance summary and potential significance to field

The authors study the role and regulation of the PHD finger protein PHF7 in germ cell sex determination in Drosophila. PHF7 is transcribed in male and female germ cells, but it is only translated in testes due to an alternative transcription start site. The authors use transgenes and tagged endogenous PHF7 to show that forced expression of PHF7 in ovaries causes loss of germ cells and production of tumors. The phenotypic effect of forced PHF7 allowed the authors to investigate several important aspects of its function. Frequency of tumors correlates with gene copies of active PHF7. The PHD domains of PHF7 are needed for its activity. PHF7 autoregulates by selecting its transcription start site. PHF7 regulates expression of genes in the germ line. Surprisingly, only a fraction of the male genes regulated by forced PHF7 in the female germ line depend on PHF7 expression in the male germ line, indicating that the action of PHF7 is depends on the cell context in which it is expressed.

The conclusions are fully supported by the data. The experiments are designed carefully, include controls, and are interpreted with care. Some conclusions are also tested by several independent means. The paper is written clearly.

Comments for the author

Some recommendations to improve the paper's impact:
It is intriguing that male genes are activated in ovaries when PHF7 expression is forced, but expression of most of these genes in male germ cells does not require PHF7. Can it be that they are only expressed in rare or transient stages in spermatogenesis, so that they might be missed in

overall transcriptomes? Is there another chromatin reader specific to testes that could compensate for loss of PHF7?

There is a correlation between amount of PHF7 and the tumor phenotype. Can the authors provide data or hypotheses for the mechanism?

The paper does not give detail about the RNAseq data. How many reads were obtained? How similar were the results between biological replicates? What were the regulated genes, including the ones regulated by PHF7 in testes and the ones that are not?

What functions are represented in the male genes that are regulated by forced PHF7 in ovaries? Do the few that are also regulated by PHF7 in testes have different functions compared to the ones that are affected by forced PHF7 in ovaries but not regulated by PHF7 in testes?

Minor comments:

Line 91-92: please state the amino acids for each PHD domain, so that one can determine whether sequences other than the PHD domains were removed by the deletion.
Line 109, Figure 1: please show an example of the agametic phenotype.
Line 116: domains should be plural.

First revision

Author response to reviewers' comments

We thank the three reviewers for investing the time necessary to provide detailed and thoughtful suggestions. We have made every attempt to fully address all comments, and believe these revisions have significantly strengthened our manuscript. Our responses, in **red**, are given in a point-by-point manner below.

In response to the reviewer's collective input, we
1) Added the requested details about our RNA-seq analysis to the text (lines 193-197 and lines 337-342).
2) Included an analysis showing the transcriptional status of the genes induced by *phf7* in wild-type female germ cells (lines 205-215, new Table S3).
3) Clarified the section describing the correlation between *phf7* copy number and phenotype (lines 157-163).
4) Modified the Figures as follows: Added Fig. S2 to supplement Fig. 2B with the addition of images of GFP channel. Modified Fig. 2 so that all images are the same size and added size bars to all panels. Increased size of images in Fig. S1. Added an image of an empty germarium to Fig. 1, as well as statistics where appropriate.

Reviewer 1 Advance Summary and Potential Significance to Field:
Establishing sexual identity of germ cells is crucial for all sexual reproduction. In Drosophila, expression of PHF7 in testis promotes male identity. Conversely, the expression of phf7 is repressed in the females by a critical regulator of sexual identity, Sex lethal (Sxl). Loss of sxl results in formation of tumors and upregulation of phf7. Ectopic expression of PHF7 independent of sxl downregulation also causes germline death and formation of tumors. This hints at the possibility that PHF7 acts downstream of Sxl to masculinize the female germline.

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3. PHF7 causes a "transcriptional reprogramming", allowing expression of "male genes" in the female germline with the caveat that the majority of the male genes expressed in the female

germ cells when PHF7 is ectopically expressed are not controlled by PHF7 in the male germ cell. Conceptual advance: As Phf7 is a chromatin reader that recruits transcriptional machinery, their model, if correct, suggests that different male specific genes are poised for expression in different tissues. Thus, presence or absence of a male specific chromatin reader can license sex specific genes in different tissues.

Reviewer 1 Comments for the Author:
Major Problems with data: While the authors have shown evidence that there is a positive feedback loop for phf7 – their other two claims are not substantiated as outlined below:
Suggestions for authors:

1A. My biggest concern is the RNA seq presented in this manuscript. I worry about comparing a phenotype that results in 70% germ cell death or 30% tumorous growth to WT ovaries.
The RNA-seq data is on mutant *nos>phf7^{EY}; HA-phf7* mutant ovaries in which the penetrance of the tumor phenotype is 70% (with 30% agametic). We have restated this observation at the beginning of the RNA seq section to avoid confusion.

1B. The overexpression of Phf7 results in accumulation of undifferentiated stages and this is compared to WT which enriches for late stage egg chambers. How can the authors control for this?
We compared our data to our RNA-seq analysis of ovaries from newborn females which are enriched for stages present in the germarium. Because ovaries from young wild-type females lack late-stage egg chambers, we reasoned that this comparison would minimize the identification of gene expression changes unrelated to the mutant phenotype. We have now included our reasoning in the manuscript. (lines 193-197).

1C. If they want to make this comparison, they should perform principle component analysis for Phf7 OE data set with data sets derived from critical stages of germ line differentiation such as GSCs and CBs that are already available. Then, they can determine which stage is the best to compare against.
To our knowledge, there are no RNA-seq data sets from purified wild-type undifferentiated germ cells. We agree with this reviewer (comment 2B) that enrichment strategies using *bam* mutants (or other mutations that prevent *bam* expression) are not appropriate for this analysis. We, and others, have documented the anomalous expression of testis genes, including the male-specific *phf7* transcript, in *bag-of-marbles (bam)* ovarian tumors (Wei *et al*. 1994; Kai *et al*. 2005; Chau *et al*. 2009; Gan *et al*. 2010; Shapiro-Kulnane *et al*. 2015; Tiwari et. al., 2020).

We therefore feel that our approach of comparing the transcriptomes of mutant ovaries with wild-type ovaries from newborn females remains the best approach at this time (as noted in comment 2B).

2A. They authors make a strong claim that a subset of genes upregulated upon the over expression of PHF7 are expressed only in the testis. To support that claim, they need to show that these genes are not expressed in the undifferentiated stages of the female germline. In addition, the authors should validate these targets either by using in-situs, antibodies or reporter lines where available. If these genes are already expressed in the female germline, then their claim falls apart.
We state that some (but not all) of the ectopically expressed genes are “are highly expressed in normal testis relative to other tissues” (line 203). This claim is based on information provided by FlyAtlas, which employs tissue-specific RNA-seq data. Validating over 100 targets one by one is not practical.

Nevertheless, we agree that it remains possible that some of the transcripts we identified as “ectopic” are normally expressed only in GSCs, and appear to be ectopic only because GSC- like cells appear to be enriched in phf7 OE mutant ovaries. To address this concern, we have taken advantage of two recently published scRNA-seq data sets obtained from adult and larval ovaries, and (as written in lines 206-213), “…we compared our gene set with genes expressed in wild-type undifferentiated germ cells obtained by single cell RNA sequencing (scRNA-Seq) of adult and larval ovaries (Jevitt et al., 2020; Slaidina et al., 2020). While we did not observe any overlap between our set of ectopically expressed genes and expressed genes in undifferentiated germ

cells isolated from adult ovaries, we did identify an overlap of 9 genes (3% of the ectopic gene set) when compared with the genes expressed in larval primordial germ cells (Table S3). Thus, the vast majority of the genes identified as ectopically expressed in tumors are induced (either directly or indirectly) by inappropriate *phf7* expression."

2B. The Salz lab has shown that bam mutants have been shown to express testis genes. To get around not using mutations, they could compare their data set to RNA seq data generated from virgin flies which enriches for stages present in the germaria.

We did compare our data to our RNA-seq analysis of ovaries from newborn females which are enriched for stages present in the germarium. (see response to comment 1C).

3A. In Figure 1, the authors find that by over expressing both WT and protein lacking PHD domain that only overexpression of full-length protein causes germline tumors. They interpret this data as evidence that PHD domain is important for function. However, to make this claim, the authors need to show that delta PHD PFH7 is in fact expressed at the protein level. I understand that an antibody is not available for PHF7. However, making a tagged version of this protein and showing that it is expressed is critical to making this claim.
We did make N-terminal GFP tagged GFP-tagged phf^{+} and $phf7^{\Delta PHD}$ transgenes with the same UASz expression vector, inserted into the same third chromosome location. Unfortunately, expressing the GFP-tagged phf^{+} construct in germ cells did not cause a mutant phenotype, suggesting that insertion of the GFP tag inactivated the protein.

We agree that it would be ideal if we could measure the amount of protein made, but we simply cannot. Instead, we have softened our language considerably. The inclusion of this data in our manuscript is necessary because we employ the defective $phf7^{\Delta PHD}$ transgene as an important loss of trans-activation control in the next section of our work. There, the reason that the $phf7^{\Delta PHD}$ transgene is inactive is not important.

3B. In lines 150-162, the authors suggest that more copies of PHF7 result in a more detrimental phenotype. However, more copies do not mean that there is more protein. The authors need to show that along with increased PHF7 copies there is a correlated increase in the protein levels.
This reviewer raises the important point that more copies does not necessarily mean more functional protein. Because we cannot measure PHF7 protein directly, we have modified the text in this section to remove mention of protein levels. The important point here is that there is a correlation between copy number and phenotype, which we exploit to generate a genetic background in which we observe 70% tumor formation.

Minor comments:

•In Figure 1B, it seems as though the magnification for each image is different. A scale bar should be included for each image.
Done.
•In line 111 the authors suggest that the germaria fail to make an oocyte. The authors should stain for oocyte markers to show that the oocyte is not specified or otherwise change their wording.
We have modified our wording to: "defined by the accumulation of germ cells in the germarium and the failure to form egg chambers with nurse cells."
•In line 114 the authors state that the flies lacking PHD domain were fertile. To truly state this, the authors should perform fertility tests on ectopically expressed flies and the flies lacking PHD in PHF7.
We have modified our wording to "produce progeny".
•In Figure 2B the authors show representative images of how ectopic PHF7 expression stimulates expression of reporter vectors. However, the images in 2B and 2C are different sizes. Fig. 2B and 2C are now the same size, and we have included size bars in all of our images.

•In Figure 2B, the authors' experimental image seems to lack any background green. If an antibody was used, one would expect some kind of background fluorescence. Please make sure all the channels were included.
All channels were included. A new Fig. S2 now shows Fig. 2B with the addition of images showing

the GFP-only channel.
•There is no scale bar for FPKM values in 4D. Fixed.

Reviewer 2 Advance Summary and Potential Significance to Field: Summary and Critique
Despite common developmental origins, oocytes and sperm undergo unique developmental programs leading to differentiation. Sexual identity in germ cells and surrounding somatic cells is a key determining factor in oocyte development. Although initiation of oocyte-specific transcriptional programs and coordinated repression of sperm-specific programs must occur to promote the oocyte program, the molecular mechanisms remain unclear.

In this study, Salz and colleagues build from previous observations that loss of the sexually-dimorphic RNA binding protein Sxl results in germ cell tumors that express Phf7, a male-specific transcription factor. Ectopic expression of Phf7 in female germ cells blocks oocyte differentiation, resulting in loss of germline stem cells and tumorous germaria. Salz and colleagues seek to determine the extent to which Phf7 can drive the male transcriptional program in female germ cells. Using novel genetic tools (including a GFP knock-in to the Phf7 open reading frame), functional studies of the Phf7 protein domains, and transcriptomic analysis, the authors present evidence in support of a model by which Phf7 in female germ cells ectopically promotes a male transcriptional program.

Overall, this well-constructed study adds exciting new molecular understanding of maintenance of sexual identity in germ cells and its links to tumorigenesis. The study advances the field by identifying genes transcriptionally regulated downstream of Phf7. Experiments are well-controlled and introduce novel reagents that will be beneficial for the field. This short report is appropriate for Development and its readers and is well-written. My specific comments/suggestions for improving the manuscript are detailed below (listed in the order in which they appear in the manuscript).

Reviewer 2 Comments for the Author: Major points
1. Results, Figure 1 (and accompanying text, lines 114-120). The authors conclude that deletion of the PHD domain renders ectopic Phf7 inactive. However, this conclusion hinges on whether the UASz-phf7[deltaPHD] actually makes a stable protein. As the authors mention, the best analysis here would be to use Phf7 antisera to make sure that a protein is actually produced. (Phf7 antisera was published in Yang et al. Van Doren 2017, Genetics 206:1939)…but perhaps these antibodies were generated against the PHD domain or don't recognize the ectopic ovarian protein? The authors should clarify their statement here.) If anti-Phf7 antisera are not available, another possibility might be to test whether the UASz-phf7[deltaPHD] causes any phenotypes in male germ cells that recapitulate phf7 mutants or fail to rescue the mutants.

We agree that it would be ideal if we could measure the amount of protein made, but we cannot. We chose not to assay function by rescue of the *phf7* loss of function phenotype in males because the phenotype is very subtle. Indeed, the mutant males start of as fertile animals (loss of function stocks are kept homozygous) and the males only become sterile when aged. Nevertheless, we feel it is important to include this information in our manuscript, because we employ the defective deltaPHD transgene as a loss of trans-activation control in the next section.

2. Results, lines 150-162. I'm confused by the authors' conclusions in this section. I agree that there is an interesting observation here: the three different experimental paradigms (phf7[deltaORF]::GFP;nos>UASz-phf7 vs. nos>UASz-phf7 in a wild-type background vs. nos>phf7[EY]; HA-phf7) yield different penetrance of the tumor phenotype. But they also give different penetrance of the agametic phenotype, suggesting stronger ectopic activity of Phf7 (rather than weaker activity, as the authors conclude). Indeed, in the truly ectopic paradigm (phf7[deltaORF]::GFP;nos>UASz-phf7, driven off the very strong nos-Gal4::VP16 but in which all regulatory information for phf7 is intact), the vast majority of the germaria (according to Fig. 1C) are agametic…wouldn't lack of germ cells (ostensibly caused by loss of GSCs) trump the tumor phenotype? I am wondering (this is pure speculation) if somehow the regulatory information is different between the three experimental paradigms, perhaps causing the phf7[deltaORF]::GFP;nos>UASz-phf7 to be more strongly expressed in larval/pupal germ cells prior to adulthood (so that GSCs aren't properly established) or in GSCs (so GSCs are lost more rapidly). Alternatively, there could be weaker expression from the nos>phf7[EY]; HA- phf7, which

might also explain these effects. In any case, the authors' statement that "We therefore conclude that the level of PHF7 protein dictates the phenotypic outcome, with the highest levels required for tumor initiation" could be experimentally tested with Western blots if suitable antibodies can be identified (see comment 1 above). If suitable reagents cannot be procured, I suggest that the authors clarify their conclusions in this section with a broader discussion.

Thank you for pointing out that the nos>phf7[EY]; HA-phf7 paradigm is not directly comparable to the other two paradigms. We have reworded this section to only compare phf7[deltaORF]::GFP;nos>UASz-phf7 vs. nos>UASz-phf7 in a wild-type background. These two genetic conditions are nearly identical, expect for the loss of the *phf7* open reading frame at the endogenous locus. It is these data that show an unambiguous correlation between *phf7*$^{+}$ copy number and phenotype.

We have clarified our conclusions by including the following statements (lines157-163): "It remains unclear why forcing *phf7* expression from a transgene in a *phf7* mutant background is toxic to germ cells, whereas in a wild-type background this same transgene allows germ cell survival and tumor formation. Irrespective of mechanism, we find that the correlation between increased *phf7* copy number and the tumor phenotype extends to a different genetic paradigm in which three full length copies of *phf7* (*nos>phf7*^{EY}*; HA-phf7*) lead to a dramatic increase in the penetrance of the tumor phenotype to 70%."

Minor Points:
1. Figure 1: Statistics are missing for panel C
Done. We used the Fisher's exact test compare the phenotypic outcomes of phf7[deltaORF]::GFP;nos>UASz-phf7 and nos>UASz-phf7. The other phenotypic outcomes do not require statistics to establish their differences from wild-type or from each other.

2. Figure 2, panels B-C aren't the same size (and are relatively small overall for the figure).
We have increased the size of B and C and included size bars in all panels.

3. Figure S1, panels in C are too small relative to the rest of the figure.
We have increased the size of the images in panel C

Reviewer 3 Advance Summary and Potential Significance to Field:
The authors study the role and regulation of the PHD finger protein PHF7 in germ cell sex determination in Drosophila. PHF7 is transcribed in male and female germ cells, but it is only translated in testes due to an alternative transcription start site. The authors use transgenes and tagged endogenous PHF7 to show that forced expression of PHF7 in ovaries causes loss of germ cells and production of tumors. The phenotypic effect of forced PHF7 allowed the authors to investigate several important aspects of its function. Frequency of tumors correlates with gene copies of active PHF7. The PHD domains of PHF7 are needed for its activity. PHF7 autoregulates by selecting its transcription start site. PHF7 regulates expression of genes in the germ line. Surprisingly, only a fraction of the male genes regulated by forced PHF7 in the female germ line depend on PHF7 expression in the male germ line, indicating that the action of PHF7 is depends on the cell context in which it is expressed.

The conclusions are fully supported by the data. The experiments are designed carefully, include controls, and are interpreted with care. Some conclusions are also tested by several independent means. The paper is written clearly.

Reviewer 3 Comments for the Author:
Some recommendations to improve the paper's impact:

1. It is intriguing that male genes are activated in ovaries when PHF7 expression is forced, but expression of most of these genes in male germ cells does not require PHF7. Can it be that they are only expressed in rare or transient stages in spermatogenesis, so that they might be missed in overall transcriptomes?
It is possible, but we have no way of discovering whether these transcripts are only expressed in rare or transient stages in spermatogenesis.

2. Is there another chromatin reader specific to testes that could compensate for loss of PHF7?
To our knowledge, no other chromatin reader specific to testes that can compensate for the loss of PHF7 has been identified.

3. There is a correlation between amount of PHF7 and the tumor phenotype. Can the authors provide data or hypotheses for the mechanism?
We could provide our thoughts on why the presence of full-length copies of *phf7* provide a survival advantage to germ cells, but at this point we feel that any discussion of this point is too speculative to be incorporated into the revised manuscript. Instead, we have included the following statement to emphasize that this observation is a conundrum. "It remains unclear why forcing *phf7* expression from a transgene in a *phf7* mutant background is toxic to germ cells, whereas in a wild-type background this same transgene allows germ cell survival and tumor formation."

4. The paper does not give detail about the RNAseq data. How many reads were obtained? How similar were the results between biological replicates?
We apologize for this omission. This information is included in the materials and methods section (lines 337-342). We write: "The number of reads generated were as follows: wildtype-1, 12,598,832 (93.7% unique), wildtype-1, 13,855,129 (93.5% unique), *phf7*-1, 12,804,022 (93.6% unique) and *phf7*-2, 12,219,346 (92.9% unique). The correlation between biological replicates was assessed using the plotCorrelation tools in DeepTools, with a 50 base pair bin size. The correlation coefficient of the biological replicates was *R*=0.99."

5. What were the regulated genes, including the ones regulated by PHF7 in testes and the ones that are not?
Again, we apologize for this omission. We have included this information in new Table S3 and new Table S4.

6. What functions are represented in the male genes that are regulated by forced PHF7 in ovaries? Do the few that are also regulated by PHF7 in testes have different functions compared to the ones that are affected by forced PHF7 in ovaries but not regulated by PHF7 in testes?
Unfortunately, the function of the majority of these genes, except the few mentioned in the text, are unknown.

Minor comments:
Line 91-92: please state the amino acids for each PHD domain, so that one can determine whether sequences other than the PHD domains were removed by the deletion.
Done
Line 109, Figure 1: please show an example of the agametic phenotype.
Done
Line 116: domains should be plural.
Fixed. Thank you

Second decision letter

MS ID#: DEVELOP/2020/192856

MS TITLE: An autoregulatory switch in sex-specific phf7 transcription causes loss of sexual identity and tumors in the Drosophila female germline

AUTHORS: Anne Smolko, Laura Shapiro-Kulnane, and Helen Salz
ARTICLE TYPE: Research Report

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks. The minor suggestions raised by the reviewers may be added in proof if you choose to implement them.

Reviewer 1

Advance summary and potential significance to field

In this study Smolko et al. have shown that:
1.Ectopic expression of Phf7 in the female germ line causes a positive feedback loop wherein PHF7 overcomes its own transcriptional silencing by promoter switching.
2.Zinc finger domain of Phf7 is required for promoting the positive feedback loop.
3.PHF7 causes a “transcriptional reprogramming”, allowing expression of “male genes” in the female germline with the caveat that the majority of the male genes expressed in the female germ cells when PHF7 is ectopically expressed are not controlled by PHF7 in the male germ cell.

Conceptual advance: As Phf7 is a chromatin reader that recruits transcriptional machinery, their model, if correct, suggests that different male specific genes are poised for expression in different tissues. Thus presence or absence of a male specific chromatin reader can license sex specific genes in different tissues.

Comments for the author

The authors have sufficiently addressed the points raised and manuscript is suitable for publication.

Reviewer 2

Advance summary and potential significance to field

In this study, Salz and colleagues build from previous observations that loss of the sexually-dimorphic RNA binding protein Sxl results in germ cell tumors that express Phf7, a male-specific transcription factor. Ectopic expression of Phf7 in female germ cells blocks oocyte differentiation resulting in loss of germline stem cells and tumorous germaria. Salz and colleagues seek to determine the extent to which Phf7 can drive the male transcriptional program in female germ cells. Using novel genetic tools (including a GFP knock-in to the Phf7 open reading frame), functional studies of the Phf7 protein domains, and transcriptomic analysis, the authors present evidence in support of a model by which Phf7 in female germ cells ectopically promotes a male transcriptional program.

Overall, this well-constructed study adds exciting new molecular understanding of maintenance of sexual identity in germ cells and its links to tumorigenesis. The study advances the field by identifying genes transcriptionally regulated downstream of Phf7. Experiments are well-controlled and introduce novel reagents that will be beneficial for the field.

Comments for the author

In the revised manuscript, the authors have addressed all of the reviewers' comments. In reading through each reviewer's comments and the author's rebuttal, I believe this manuscript is significantly improved and now suitable for publication with minor revisions. My only remaining issues are:
1. The immunofluorescence images in Figures 1 and 2 have extra scale bars in each panel.
2. I would like to see the information in Fig S1 and Fig S2 incorporated into the main figures, as these control experiments are critical for interpreting the data presented in the main figures.
3. In Figure 4A, there is a backwards "Phf7" label on the mRNA diagram.
4. In Figure 4C, the legend for Z-score is missing y-axis values. (Also should it say "raw" Z-score instead of "row"?)

Reviewer 3

Advance summary and potential significance to field

Smolko et al show that forced expression of PHF7 in Drosophila ovaries causes loss of germ cells, production of tumors, and abnormal gene expression. Gene expression abnormalities include induction of a few testis genes, and also genes that are not expressed in testes. This latter result shows that PHF7's action depends on the cell context in which it is expressed. Finally, Smolko et al. define PH7's functional domains, and show that it autoregulates.

Comments for the author

As prior Reviewer 3, I was already very positive about this paper, but I think the modifications improved it further. I appreciate the authors' responses to my requests, including adding details of the RNAseq data, coordinates for the deletion, and names of the regulated genes. Their added statement to address my comment 3 is also fine, and I understand and am convinced by their responses to my other comments. Regarding my comment 1, might it be useful to mention around line 223 that of course genes expressed only very transiently or rarely during spermatogenesis might have been missing from the reference dataset? This is up to the authors to decide.