The Blimp-1 transcription factor acts in non-neuronal cells to regulate terminal differentiation of the *Drosophila* eye

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MS TITLE: The Blimp-1 transcription factor acts in non-neuronal cells to regulate terminal differentiation of the Drosophila eye

AUTHORS: Hongsu Wang, Carolyn A Morrison, Neha Ghosh, Joy S Tea, Gerald Call, and Jessica E Treisman

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.

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. I would particularly encourage you to improve the phenotyping of the mutant and attempt to shed more mechanistic light on it. 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.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. 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.

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

During terminal differentiation, tissues must orchestrate precise patterns of transcription in space and time that build and shape tissue to its function. Substantial and informative effort, much contributed by this PI's lab, has revealed much of the genetic circuitry that specifies cell fates in the Drosophila eye; relatively less insight exists as to how these cells, once specified, effect programs of terminal differentiation. Following upon their recent demonstration that the zinc finger transcription factor Glass, long thought to specify photoreceptor cell fating, also promotes differentiation of non-neuronal cells in the eye, the authors investigate Blimp-1, an ecdysone-induced transcription factor known for several roles, including temporal regulation of chitin secretion and a predicted regulator of several Glass target genes. The authors report a number of phenotypes arising later in differentiation, notably including the intriguing observation that the normally biconvex facet lens becomes plano-convex in response to Blimp-1 loss. Together, several observations here support a role for Blimp-1 in terminal eye differentiation.

Comments for the author

Although Blimp-1 cornea lacks the sharp hexagonal valleys that bound wild type facets and so are “without clearly demarcated corneal lenses”, the normal hexagonal IOC grid of attachment to the cornea is nonetheless plain in Fig. 1B. Eye bristles are approximately normally placed, consistent with normal pattern specification. Bristles are partially submerged by extra material filling in the valleys between facets. Fig. 2A indicates this material includes chitin, which is normally deposited only above the cone cells and primary pigment. As figured in Stahl et al., 2017’s electron micrograph, corneal lens chitin assembles into layers that do not continue above IOCs, yielding a biconvex curvature. An equivalent electron micrograph of Blimp-1 showing chitin layers continuing above could support that the plano convex lens arises in a failure to repress chitin deposition in IOCs.
For the measurement of lens focal lengths in Fig. 2, the agreement of control focal lengths with Stavenga 2003 suggests the measurements were done accurately, but lens measurements should be shown with Supplemental Fig. 4. What were H1, H2, W, and T values? The scale bar in Fig. 2E,F should be checked as it suggests a lens larger than that reported in Stavenga, 2003, 16u diameter, 8u thick.
In Fig. 4A,B, is the graded CBD stain, prominent in the insets, a dye penetration artefact or a chitin gradient?
This could be checked with Calcofluor White, a strongly fluorescent chitin-binding small molecule that fully penetrates and stains dense lens chitin in corneal whole mounts.
In Fig. 4E’, it would be useful to indicate which nuclei are the higher order pigment cells. In Fig. 4D, the PR PPC plane, the ommatidium just left and below center contains a trio of GFP+ photoreceptors and a GFP- primary to the northwest of the cluster. This photoreceptor cluster also appears in 4E, the plane designated as PC. Since the IOCs are still sorting out at this stage (Ecad in C’), their nuclei have not well stratified into the distinct layers as in H, H’, making it challenging to identify PCs.
Interpretation of Fig. 5D’ as showing fused rhabdomeres needs to be made with caution. The plane of this section intersects eye curvature such that only ommatidia to the lower right are cut tangentially at a distal plane where rhabdomeres normally converge immediately below the cone cells. In the upper left quadrant of D’’’ rhabdomeres in GFP+ tissue, although oblique, appear normally separated.
How were 2° cells identified in Fig. 7B”? At this stage, primary pigment cells and cone cells dominate the distal surface of the retina. IOCs, with their nuclei and most cytoplasm proximal (e.g., Fig. 4H”), are reduced to thin profiles, which can be seen in GFP channel as hexagonal outlines in the upper left corner of 7B. The ring-like slbo-lacZ puncta in the lower half of the field are more consistent with the proximal curtains of the primaries which embrace the distal photoreceptor cluster. A panel showing a deeper plane of this mosaic would be more diagnostic for

2° cells. In Fig. 7F, there appears to be substantial CBD staining throughout the 54>UAS-slboHA relative to Fig. 2A, is this significant?
Since ommatidial depth grades across the eye, e.g. with longer ommatidia at the anterior, if a measure of 90 ± 2.7 μm, n=10, is specified for mutant retina depth (p. 8), it would be useful to note where the depth measures were made.

Reviewer 2

Advance summary and potential significance to field

Comments for the author

Review of Wang et al

In this paper, the authors describe the role of the Blimp-1 transcription factor in fly eye development. They find that Blimp-1 acts in 2ndary and tertiary pigment cells to promote bi-convex lens and acts in cones to enable extension of PR rhabdomeres. They go on to find that ecdysone is required for timing of blimp expression. Additionally, Blimp-1 represses *slow border cells (slbo)* and overexpression of Slbo recaps effects of Blimp-1 mutants. They also show that Blimp-1 is transiently expressed.

Overall, this is an excellent description of the role of Blimp-1 in fly eye development. As described below, the authors should provide some data/quantification for experiments that they've already completed, analyze gene expression data that is readily available, and make minor text and figure changes. With these changes, the paper will be ready for publication in Development.

Detailed comments

1. Figure 1. Figure 1 would benefit from adding a diagram describing the structure of the eye as an introduction. The authors show the rescue of the Blimp-1 12 mutant allele, but not the mutant phenotype on its own – please provide an image of this mutant phenotype. K'' is in a different focal plane as the other images. Please provide a separate set of panels at 46 hours of all channels in this focal plane.

2. Please mark the clone boundaries for clarity in all mutant clone experiments.

3. Show the absence of Blimp-1 expression at 72 hours. The authors argue that Blimp-1 plays a role early to regulate gene expression and show expression through early pupation. They state that expression ceases by 72 hours but don't show this timepoint ("data not shown"). As this is an important part of their conclusions, the authors should include an image that shows the ceasing of Blimp-1 expression.

4. Be consistent with IHC colors/simplify labeling when possible. The authors use different colors for different antibody-based imaging approaches. Please alter the images to generate as much consistency as possible with the IHC images. Along these lines, I recommend using generic labels for reporters (reporter instead of GFP, tomato, etc.) and genotypes (wild type/mutant instead of detailed allele info) when possible to make it easier for the reader. In particular, reading GFP in a non-green color (or any other similar reporter) makes it harder for the reader.

5. Figure 2. Fig. S4 should be moved to figure 2 as it greatly aids in understanding the system. Fig. 2G should be brightened as it did not print well.

6. Figure 3. Please quantify rhabdomere length, nuclei positions, and feet positions. Also please provide a zoom in on E''.

7. Figure 4. Ecydosone plays many roles in many cell types to control temporality of development. One suggested experiment (not required) would be to express EcRNAi under cone and/or pigment cell specific drivers to show the cell autonomous effects. Also, please provide the genotypes in fig 4C-G. Also, please state that ecad marks cell boundaries in the figure legend

and/or text.

8. Figure 5 – The data in figure 5 does not seem particularly critical for the main story in the paper and should be moved to the supplemental materials. The authors make some conclusions about how Blimp-1 regulates gene expression based on this experiment but it is challenging to interpret as the timing and levels generated in this experiment are unclear.

9. Figure 6. Analyze and identify genes whose expression changes around the 2 day pupal timepoint – The authors make arguments about the temporal nature of Blimp-1 regulation based on the peak expression of target genes in Fig 6E-F. However, this does not appear to be the best analysis. The authors should identify Blimp-1 target genes whose expression normally changes (stably increasing or decreasing) at the ~2 day timepoint. These genes would provide a better argument for temporal regulation by Blimp-1. Additionally, the authors should simplify the paragraph about lens proteins.

10. Remove/edit binding site arguments. Often, the authors state that there are binding sites for Blimp-1 or other TFs in target genes. They use these sites as an argument for direct action of Blimp-1. However, binding sites can be found in many genes, especially when one considers lower affinity binding sites. The authors would have to carry out Blimp-1 chip-seq to make statements about direct action of Blimp-1. I think that asking for Blimp-1 chip seq is beyond the scope of this paper and is not required for publication. Rather, I suggest that the authors remove or significantly truncate/tone down the discussion of these binding sites.

Reviewer 3

Advance summary and potential significance to field

In this manuscript, Wang et al investigate the role of the transcription factor Blimp-1 in retinal differentiation in Drosophila. This factor is known to act as a late component of the ecdysone-induced transcriptional cascade required for the differentiation/maturation of precursor cells. Blimp-1 is known to be active in many different cell types regulating a plethora of genes (for example Blimp-1 promotes maturation of tracheal cells by regulating chitin deposition or F-actin organization doi: 10.1534/genetics.118.301444). Here the authors find that Blimp-1 expression is expressed dynamically in all the different retinal cells during pupal stages. Blimp-1 inactivation leads to defects in the curvature of the outer surface of corneal lens due, a phenotype mediated by differentiation defects in secondary and tertiary pigment cells. In addition, the morphogenesis of photoreceptors is affected by disruption of cone cell differentiation.
The authors attempted to identify which mis-regulated genes are responsible for the observed defects. For this purpose, they perform various transcriptomic analysis upon mis-expression or knockdown of Blimp-1 to find that a large number of terminal differentiation genes are deregulated such as cuticle proteins that are possibly involved in shaping the lens.
Finally, the authors concentrate on the gene slow border cell (slbo) that is strongly up-regulated in the absence of Blimp-1. Interestingly, slbo mis-expression can phenocopy Blimp-1 down regulation although co-inactivation of slbo and Blimp-1 does not rescue the phenotype.
All together, this analysis confirms that Blimp-1 regulates many different genes involved in terminal differentiation and identifies two different functions for Blimp-1 in two different cell types of the retina. The transcriptomic analysis provides lists of deregulated genes but, does not provide information about the cell-specific cellular transcriptional response to Blimp-1 inactivation.

My opinion is that this manuscript is solid in that the phenotypes are well described and the experiments support most of the conclusions. It confirms that Blimp-1 acts in many cells and regulates many terminal differentiation genes in a cell-specific manner. However, the mechanistic insights are limited and for example, the study does not really identify the mechanisms responsible for defects in the curvature of the lens or photoreceptor morphogenesis. One aspect that is not addressed is the mode of action of Blimp-1 and how the specificity of its targets is achieved in the different cell types.

Comments for the author

Major points:
- The presence of Sallimus (Sls) in cone cell feet is intriguing as sls is supposed to be exclusively expressed in muscle cells. The expression of this gene in cone cells has not been convincingly documented by previous studies. Moreover Sls does not seem to be expressed in cone cells from single-cell data present from the fly cell atlas (https://flycellatlas.org/#data). To show that Sls staining in cone cells is not an artefact, the author should check that this staining is lost upon expression of sls RNAi.
Is sls downregulated in the transcriptomic analysis upon Blimp-1 inactivation?
It would be also interesting to understand how sallimus down-regulation contributes to the defects in photoreceptor morphology.
In addition, in order to better characterize how loss of Blimp-1 affects cone cells, the authors could take advantage of the single-cell data available from the "fly cell atlas" to match with their transcriptomic analysis and identify other cone cells markers to be tested in the Blimp-1 mutant condition.
- Figure 7a: Elav seems to be upregulated upon loss of Blimp-1 which contradicts the stainings in Figure 1G-J. Could you please clarify this point?
- Is Blimp-1 expressed in larval eye imaginal discs?

First revision

Author response to reviewers' comments

We thank the reviewers for their appreciation for the significance of our findings and for their critical comments, which have enabled us to significantly improve our manuscript. Our detailed responses to their comments are described below:

Reviewer 1:
1) The reviewer suggested that the plano-convex corneal lens phenotype caused by loss of Blimp-1 function could reflect excessive chitin deposition above inter-ommatidial cells, and asked us to examine this by electron microscopy. We have added electron micrographs of ommatidia from a control eye and an eye in which Blimp-1 is knocked down in secondary and tertiary pigment cells (Fig. 2I, J). Our images do not show chitin deposition above these pigment cells in the absence of Blimp-1; rather, the tips of the pigment cells are level with the outer surface of the corneal lens, consistent with the plastic section shown in Fig. 2F. In addition, the cross-sectional area of the corneal lens does not appear to be increased. Thus we do not believe that the change in corneal lens shape results from a failure to repress chitin deposition.

2) The reviewer asked for the corneal lens measurements that were used to calculate the focal length values shown in Fig. 2O. We have added these in a source data file. We have also corrected the scale bars for Fig. 2E, F.

3) The reviewer recommended using Calcofluor White to label chitin to determine whether the graded CBD staining we observe in corneal lenses is a penetration artefact. We thank the reviewer for the suggestion and now show Calcofluor White staining in Fig. 2A, G and in insets in Fig. 4A, B. There is some variability in staining with both CBD and Calcofluor White; because we do observe staining throughout the corneal lens in some samples, we would not argue that there is a chitin gradient within the corneal lens.

4) The reviewer asked us to indicate which nuclei corresponded to higher order pigment cells in Fig. 4E. We have replaced this panel with a confocal section that more clearly shows these pigment cell nuclei, and have marked them with arrows.

5) The reviewer thought that the plane of the section we showed in the previous Fig. 5D made it difficult to be certain that Blimp-1 overexpression caused rhabdomere fusion. We now use this section only to show TrpL staining, and have added a new image cut at an angle at which the

rhabdomere defects are more clearly visible (Fig. S9E). We have also added an image showing that excessive Chp staining in Blimp-1 overexpressing ommatidia is already visible in the mid-pupal retina (Fig. S9G).

6) The reviewer thought that the cells identified as secondary pigment cells in Fig. 7B (now 6B) might be the proximal curtains of primary pigment cells. We have replaced this panel with two separate focal planes, one showing cone and primary pigment cells (B, B') and the other showing secondary pigment cells (B'', B'''). We have also replaced Fig. 7F (now 6F) with an example that has less background staining with the CBD probe.

7) The reviewer asked at which position in the retina we measured ommatidial depth. We have clarified that this was measured at the anterior, where the depth is greatest (p. 8).

Reviewer 2:
1) The reviewer thought that Figure 1 should include a diagram of the structure of the eye. We took this to refer to the structure of the pupal retina, shown in panels H-M, and added a diagram of this stage as Fig. 1F. The reviewer requested that since Fig. 1E shows rescue of the *Blimp-1*^{12} allele, we should show a similar image of the unrescued *Blimp-1*^{12} mutant. We have replaced the *Blimp-1*^{17} image previously shown with *Blimp-1*^{12} (Fig. 1D). We note that these two alleles have the same CRISPR-generated deletion and their phenotypes appear identical. The reviewer also asked for a set of panels at 46 h APF shown at the same focal plane as Fig. 1K'' (now L''). We replaced L'' with the cone cell plane shown in L, and included a set of panels showing the primary pigment cell and photoreceptor plane as Fig. S1B.

2) The reviewer asked us to mark the clonal boundaries in panels that do not include the clonal marker. We have now done so in Fig. 1H, J, L, Fig. 3A, D, E, Fig. 4C-G, Fig. S1B, Fig. S2B, Fig. S3A, B, Fig. S6B, and Fig. S8A.

3) The reviewer asked us to show the absence of Blimp-1 staining in the 72 h APF retina. We have added these data as Fig. S1C.

4) The reviewer asked us to use the same colors for all antibody-based images. We have now converted all three-channel images to red/green/blue and two-channel images to magenta/green, made the colors used for reporters correspond to their fluorescent wavelengths, and tried to simplify the figure labels where possible.

5) We have moved Fig. S4 to Fig. 2M, N as requested by the reviewer. We have replaced Fig. 2G with an image labeled with Calcofluor and CBD for better visualization of the corneal lenses.

6) The reviewer asked us to quantify rhabdomere length and the position of photoreceptor nuclei (we do not claim to see a change in the position of cone cell feet). We have added these quantifications in Fig. 3F, G.

7) The reviewer suggested that we could express *EcR* RNAi with cone or pigment cell specific drivers to characterize its cell-autonomous effects. We have tried these experiments, but did not see phenotypes, probably because the drivers are not strong or early enough to fully deplete *EcR*. We have added the genotypes to Fig. 4C-H, and stated in the figure legend that Ecad marks apical cell membranes.

8) The reviewer thought we should make Fig. 5 supplementary. It is now Fig. S9.

9) The reviewer suggested that instead of categorizing genes by their time of peak expression in the previous Fig. 6E, F, we should quantify the number of genes that are stably increasing or decreasing their expression at 48 h APF. We have replaced this analysis as requested (now Fig. 5E), We find that of the genes that are upregulated by *Blimp-1* knockdown, more are increasing than decreasing their expression at this time, suggesting that Blimp-1 functions to delay this increase. We have also simplified the paragraph about lens proteins on p. 12, as requested.

10) The reviewer thought that we should remove or edit our arguments about predicted Blimp- 1 and Slbo binding sites. We have left our analysis in Fig. S10A, B, but put less emphasis on it in the text.

Reviewer 3:
1) The reviewer found our paper solid and the conclusions well supported by the data, but would have liked to see more insight into the mechanisms responsible for the defects in corneal lens curvature and photoreceptor morphogenesis, and into how Blimp-1 regulates different targets in different cell types. We believe that our paper already makes a significant contribution by identifying several phenotypes caused by loss of *Blimp-1* and determining the cells in which it is required for each phenotype, describing its expression and regulation by ecdysone signaling, and characterizing its downstream target genes. Understanding the mechanisms would be a major additional undertaking that is beyond the scope of this paper. We have made some efforts to narrow down potential mechanisms in this revision, for instance by adding electron micrographs showing the effects of knocking down *Blimp-1* in secondary and tertiary pigment cells on the corneal lens (Fig. 2I, J), and by identifying the Blimp-1 target gene *trol*, which encodes an extracellular matrix protein localized to cone cell feet, as a possible mediator of the effect on rhabdomere extension (Fig. S8).

2) The reviewer was surprised by the presence of Sallimus in cone cell feet. This has previously been observed both in RNA-Seq data from sorted cone cells at several stages (Charlton-Perkins et al., 2017) and by antibody staining (Morrison et al., 2018). We now also show that expression of *sls* RNAi removes Sls staining from cone cell feet, and that Sls colocalizes with the known cone cell marker Fasciclin III in apical regions (Fig. S7). *sls* indeed shows reduced expression in retinas expressing *Blimp-1* RNAi (Table S1). However, *sls* in cone cells is not necessary for normal photoreceptor rhabdomere extension, as we now show in Fig. S7B.

Charlton-Perkins, M.A., E.D. Sendler, E.K. Buschbeck, and T.A. Cook. 2017. Multifunctional glial support by Semper cells in the *Drosophila* retina. *PLoS Genet*. 13:e1006782.
Morrison, C.A., H. Chen, T. Cook, S. Brown, and J.E. Treisman. 2018. Glass promotes the differentiation of neuronal and non-neuronal cell types in the *Drosophila* eye. *PLoS Genet*. 14:e1007173.

3) The reviewer suggested looking for other Blimp-1 target genes that are expressed in cone cells and might contribute to the photoreceptor phenotype. We identified *trol* as a gene that was previously shown to be enriched in cone cells (Charlton-Perkins et al., 2017) and is reduced by *Blimp-1* RNAi (Table S1). We confirmed that Perlecan, the product of the *trol* gene, is expressed in cone cells, localized to their feet, and down-regulated in the absence of *Blimp-1* (Fig. S8A, B). Knocking down *trol* causes rhabdomere shortening, suggesting that its reduction may partially explain this effect of *Blimp-1* loss (Fig. S8C, D).

4) The reviewer commented that Elav seemed to be upregulated in *Blimp-1* mutant clones in the image shown in Fig. 7B. This impression was due to the tangential nature of the section; the image we now show in Fig. 6B'' shows that loss of *Blimp-1* does not affect Elav expression.

5) The reviewer asked if Blimp-1 was expressed in larval eye imaginal discs. It is not, and we now show this in Fig. S1A.

Significant changes are highlighted in the text. In addition, supplementary figures 1, 7 and 8 are new, and supplementary figure 9 is a modified version of the former main figure 5.

Second decision letter

MS ID#: DEVELOP/2021/200217

MS TITLE: The Blimp-1 transcription factor acts in non-neuronal cells to regulate terminal differentiation of the Drosophila eye

AUTHORS: Hongsu Wang, Carolyn A Morrison, Neha Ghosh, Joy S Tea, Gerald Call, and Jessica E Treisman
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.

Reviewer 1

Advance summary and potential significance to field

See prior review.

Comments for the author

With this revision the authors have satisfactorily addressed matters raised in my prior review.

Reviewer 2

Advance summary and potential significance to field

The authors found a role for the transcription factor Blimp-1 in differentiation of photoreceptors in the fly eye.

Comments for the author

The authors have addressed my concerns and this excellent paper is now ready for publication in Development.

Reviewer 3

Advance summary and potential significance to field

In this revised version, the authors have clarified my main technical concern about the expression of Sallimus in cone cell feet. In addition, they have made nice additions to the manuscript with the characterisation of the Blimp-1 mutant by electron microscopy and characterisation of the role of another potential Blimp-1 target, trol, in rhabdomere shortening. They have also improved the presentation of the data overall.

Comments for the author

All together, these revisions significantly improve the manuscript at multiple levels, making it a solid contribution to our understanding of how the fly retina is built during development, and ready for publication.