Inter-plane feedback coordinates cell morphogenesis and maintains tissue organization in the Drosophila pupal retina

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MS TITLE: Orthogonal coupling of a 3D cytoskeletal scaffold coordinates cell morphogenesis and maintains tissue organization in the Drosophila pupal retina

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Apologies for the length of time it has taken to make a decision on your manuscript. However, 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 the topic of your work, but they consider that aspects of the phenotypic analyses are not sufficiently robust to justify the conclusions that you make and that further experiments are needed to explore determine how and why the phenotypes arise.If you are able to address these concerns and revise the manuscript as suggested, I will be happy receive a revised version of the manuscript. Please also note that Development will normally permit only one round of major revision. If it would be helpful, you are welcome to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating your plans for addressing the referee’s comments, and we will look over this and provide further guidance.

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

Authors note "Prior phenotypic analyses showed that Abl is required for multiple aspects of the photoreceptor terminal differentiation program and that its loss perturbs ommatidial organization and retinal pattern (Bennett and Hoffmann, 1992; Henkemeyer et al., 1987; Henkemeyer et al., 1990; Kannan et al., 2014; Singh et al., 2010; Xiong and Rebay, 2011; Xiong et al., 2013)." They further note that evidence shown in the paper suggests "Abl function is required at the cellular level to produce the specialized shapes, structures and connections of the photoreceptors and IOPCs."

Comments for the author

It is unclear what the authors regard as the cause of photoreceptor “falling” in abl mutants. This fundamental is required to understand how phenotype rescue may operate. One possibility is that the wave of R cell axon shortening that draws the lamina beneath the retina (Langen et al., Cell 2015) exerts tension that pulls mutant R cells, poorly connected to each other via defective cell-cell junctions, out of the epithelium. Restoration of R cell junctions via elav-Gal4 driven Abl could promote a larger R cell assembly harder to pull through the basal ring. Conversely, stronger IOPC junctions produced by LL54-Gal4 driven Abl might promote a stronger basal ring, again hindering R cells from being pulled through the floor. Phenotypes and rescues shown here may be traceable to the well-established role of abl in cell-cell junctions.

Although it is possible that “The gradual improvement in regularity of ommatidial hexagonal packing suggested that IOPCs continuously optimize their contacts within the apical network, allowing them to recover a more uniform tension distribution after the disturbance of the falling photoreceptors moved away from the apical toward the basal side”, the re-appearance cone cells previously missing from the apical plane in Fig. 2D to form the quartets seen in 2F would require a dramatic reorganization of junctions, including ones that recover the seriously misplaced bristle positions in 2D. Given the pupal lethality observed in abl mutants, is it possible that animals surviving to 100% are less impacted and show better organization? Direct demonstration that cone cells are resurfaced in such orderly fashion is needed.

The videos demonstrate the difficulty of live animal imaging but show little interpretable change over the time course imaged. The color overlays can be problematic, e.g., in Video S2, at 75. 80 and 85 min, the left side falling photoreceptor is shown as co-planar with the green photoreceptor in the Basal view when they do not overlap in the Lateral view.

Fig. 2L is oblique and does not show the wt fenestrated membrane, which is indicated for abl in 2N. This could confuse readers.

What is shown in Fig. 4 G,G’? Labeled as abl null, these ommatidia appear to have reasonable early rhabdomeres.

How is the “non-enrichment” of Ena in Fig. 4F to be interpreted? Per Fig. S2E, there is little F-actin in IOPC feet at this stage.

Readers following the authors’ previous work on Abl in the eye would benefit from guidance in connecting their 2011 Dev. Dynamics observation that; “Abl does not appear to be expressed in the cone or other accessory cell types” and the current “Abl expression was detected in all retinal cell types, with enrichment in the IOPC and photoreceptor cytoskeletal and junctional domains that define the 3D structural scaffold (Figure 3 and Figure S3).” Likewise, the abl mutant cluster 2011, Fig. 4F, is quite different from the one shown here in Fig. 1G. How does Fig S1 here, showing photoreceptor fate is not changed in abl null eyes square with their Development 2013 report: “Here we show that Abl is required for photoreceptor cell fate maintenance, as Abl mutant photoreceptors lose neuronal markers during late pupal stages.”? In 2011, they note “Rhabdomeres Fail to Generate in abl Mutant Photoreceptors” but here report “fragmentation and misalignment of abl mutant rhabdomeres.” Although it is stated that the R cell apical surfaces are here referred to as rhabdomeres for “simplicity”, this muddles the failure of abl R cells to form one of the major mechanical elements of the retina.

Reviewer 2

Advance summary and potential significance to field

In this study, Sun et al., explore the role of the F-actin effector kinase Abl in morphogenesis, using the fly retina as a model epithelium, where cells need to interact to form a functional visual system. Overall, I think the topic is very interesting and the model system is very powerful to study how different cell types interact in morphogenesis. From their genetic experiments and imaging of live and fixed preparations, they conclude that Abl functions in how retinal cell types coordinate their morphogenesis to generate a functional retina. As they had shown before (Xiong 2011), Abl is required in the photoreceptors for normal morphogenesis, which in this current study they suggest depends on ena. Abl also functions in the interommatidial cell for their normal morphogenesis. By selectively perturbing morphogenesis in these cell types, they show their morphogenesis/arrangement is interdependent. The rescue experiment in Fig6j-k is particularly striking. In my view this rescue exemplifies the novel key finding of the work and this should be of broad interest.

Overall, the quality of the imaging data is very good, and the experiments are well executed. However, the work suffers from a lack of quantification, some missing controls and at time overinterpretations – especially regarding the inferred linked between “ring shape” and tension in cells (please refer to comments below).

Comments for the author

I have specific comments that the authors might want to consider, which I think would strengthen some of their key conclusions. I hope the authors will find some of these comments useful.

- Fig1D-E and Pp4 ”in wildtype, the photoreceptor nuclei clustered in a tight row just below the retinal surface” It should be noted that the nuclei of the R8 cell are basal. In the time lapse (1G), it would be useful to clarify how they identify the “falling photoreceptors”. As far as I can tell they are not using any marker of cell identity in these movies (e.g. chp>GFP)?

- There are a number of statements that are vague, which make it difficult for the reader to understand the various processes examined in the study. For example: “rhabdomeres appeared disorganized” What do the authors mean by this? Length? Position? Thickness? A more quantitative analysis would help.

- “Together, these observations suggest that the distinct shapes and spatial arrangement of the photoreceptors and IOPCs is critical to the tissue’s ability to withstand morphogenetic change and maintain integrity.” Based on the genetics and imaging pretend at this point of the paper, I think a more accurate conclusion would be that Abl is broadly required in retinal cell morphogenesis.

- “Scaffold alignment and connections are maintained throughout the elongation phase despite extensive remodeling of the cellular structures.” Clarifying what is meant here by remodelling of the cellular structures would be useful. Globally all retinal cells elongate. The rhabdomere becomes more organised (ie cylindrical) etc?

- “leaving uneven spacing between neighboring ommatidia” This aspect of the phenotype is not easy to appreciate from 1G.

- Including the rhabdomeres in the scaffolding axis implies that rhabdomere elongation drives the thickening of the retina. I am not sure that evidence that this is the case exists in the literature. During photoreceptor elongation, membrane is equally apposed at the apical rhabdomere, the sub-apical membrane and at the actomyosin-rich adherens junction, which could equally drive elongation? Is the authors model that the all-ena axis drives rhabdomere morphogenesis (ie microvilli) and that this somehow drives elongation mechanically? What is the evidence for this model?

- As they fall, the photoreceptors may further perturb scaffold structures and connections. How do the authors relate the fall of these cells to their previous work showing abl loss of function impairs apical-basal organisation and adherens junction remodelling in these cells?

- Fig.2, 75% pd does not look wild type, probably due to mounting issues. Since the authors compare phenotype with the wildtype, they should provide a better picture for the 75% time point.

- In the absence of experimental evidence on cell tension, I do not think the author can state “suggested an anisotropic tension distribution” (Figure 2D vs. 2A).

- “some cone cells dropped sub-apically” I could not find the data supporting this comment. Perhaps a staining for a cone cell marker could be included?

- By 100% p.d., apical network pattern was improved, as indicated by the more regular ommatidial shapes and IOPC and cone cell apical profiles. This improvement is difficult to see from 2E to 2F. I am not sure I see the amelioration in the quantification presented in 2G.

- “F-actin localization outlined a recognizable radial pattern of IOPC feet in abl mutant retinas (Figure 2D’ vs. 2A’) although heterogeneity in the central rings suggested tension was unevenly distributed across the plane”. The link between central ring diameter and tension is weak and not supported by any experimental evidence (e.g. tension measurements) in the paper or literature. It might be better to quantify and comment on the F-actin staining, which seem reduced in the abl mutant, and the long axis of cells, as some seem much more contracted than in the wt. Based on what is shown in Fig1,wherre the photoreceptor slide towards the brain, I think that the authors should consider that it is possible that the increased diameter of the ring could be due to the photoreceptors simply occupying part of that space. This would have nothing to do with tension in the IOC basal feet.

- "the radial alignment of their F-actin bundles, their connections to the central rings, and the underlying ECM all appeared increasingly disorganized and variable." Quantification would be very useful here.

- “Retinal depth was significantly reduced relative to wildtype (Figure 2N vs. 2L; Figure 2J). Together these phenotypes suggest Abl function is required at the cellular level to produce the specialized shapes structures and connections of the photoreceptors and IOPCs”. The authors should ensure that this falling phenotype is not already seen early in retinal morphogenesis as this would also lead to photoreceptors trapped below the fenestration membrane, but would have little to do with pupal morphogenesis per se.

- “rhabdomere organization (Figure 4A-4B’)”. The authors should clarify what they mean by organisation. The relative positioning of the rhabdomere within the ommatidium? Their length or their width? The amount of F-actin they contain?

- Are the panels projections of confocal sections? Ideally, these images should be accompanied with quantification of the number of apical(?) elav nuclei and number of detected rhabdomeres.

- Fig4E looks like an earlier pupal stage, preceding photoreceptor elongation. It would be good to show this staining a bit later, when the rhabdomere/subapical membranes of these cells are better separated. A subapical marker (e.g. aPKC) would also help here.

- “Abl deploys Ena-dependent regulation in the photoreceptor rhabdomeres but acts via Ena-independent mechanisms in the IOPC contractile feet”. There is a faint signal at the basal surface for Ena (4F). From the text, the author’s implied view is that this is background noise. It would be good to check this, by generating ena clones stained with the Ena antibody.

- Fig 4H-I needs n numbers and quantification, and needs to include the abl controls (elavgal4, abl and LL54gal4, abl), and the LL5gal4>enaRNAi on its own.

It would be good to also compare the expression of Gal4 under the elav promoter versus LL54: duration timing and strength of expression, using a UAS-gfp, for example, as this could also explain differences in rescue. Another approach might be GMRgal4, elavgal80, compared to GMRgal4 alone.

- (Fig5) "Non-autonomous rescue of basal network pattern was also evident, with reduced variation in ring sizes indicating a more uniform distribution of tension." Please see previous comment related to Fig.2A', 2D'. Perhaps this might not be linked to tension, but instead to the photoreceptor defenestration phenotype.

Reviewer 3

Advance summary and potential significance to field

This manuscript by Sun and colleagues identifies a role for Abl in establishing and maintaining the three-dimensional (3D) stereotyped organization of the Drosophila pupal retina. While 2D supracellular cytoskeletal structures are well known to coordinate morphogenetic processes, here the authors propose that a 3D supracellular structure is essential for patterning and organizing the different cell type in the retina.
Additionally, they identify an intercellular feedback mechanism where different cell types can correct and rescue patterning defects in another cell type and vice versa.

The authors use careful 3D imaging of developing pupal retinas to characterize the effects of loss of abl on the retinal epithelium and its 3D organization. The authors determine that these defects can be rescued in a cell type specific manner, where driving expression of Abl in either photoreceptors or IOPCs alone can rescue the entire epithelial 3D organization.

Finally, the authors suggest that the cytoskeleton and cell-cell junctions of different cell types in the retina (photoreceptors and IOPC) together generate a 3D cytoskeletal scaffold that act to generate tissue-level organization of the retinal epithelium. While I find this hypothesis exciting – I think that the evidence of the 3D cytoskeletal scaffold needs improvement. Instead, in this study, the authors carefully showed the necessity and sufficiency of Abl in establishing and maintaining the 3D organization of the retinal epithelium, however the only downstream effector they assay is Ena and there is little evidence to support how this "scaffold" is remodeled during development or how Abl promotes "mechanical connectedness" of the scaffold. To account for this mismatch, my suggestion is for the authors to either:

- Revise the manuscript to emphasize the role of Abl in the 3D organization of the epithelium.

- Or do more experiments that focus on identifying components of the cytoskeletal scaffold and specifically how Abl promotes "mechanical connectedness". For example, look and query more directly at how the cytoskeleton is connected across cells via junctional components.

Comments for the author

1. Figure 1B – rather than a 3D rendered view of the retinal epithelium two schematics that depict the majors steps on the patterning and elongation phase, and final organization of the epithelium may be more helpful to readers, especially those unfamiliar with the retinal system and its morphogenesis.

2. "To bridge cellular and tissue scale analyses, we further conceptualized the specialized cytoskeletal domains that organize the apical, basal and longitudinal planes as a 3D structural scaffold (Figure 1F')" (p5).

In the absence of the context from Figure 2, I did not understand this phrase, nor did Figure 1F' help me understand the concept of a 3D scaffold. At this point in the manuscript, I am convinced that Abl is an important player in epithelial organization, but describing it as a 3D cytoskeletal scaffold at this point seems premature. Rather, I would suggest removing that sentence and describing the cellular and tissue-defects as done already.

Additionally, the schematic of Figure 1F' did not help me understand what the 3D cytoskeleton scaffold is.

3. Figure 4 + text section – describe that elav-Gal4 drives expression in photoreceptors and LL5-Gal4 drives expression in IOCPC. This is explained clearly in the next section (Figure 5) but would help the readers that are unfamiliar with the Drosophila retinal system understand the experiment better if presented earlier in the manuscript text.

4. Figure 4 – add basal images of ablnull with Ena and F-actin staining, it is hard to tell if Ena is more "basally dispersed" (p7) without the comparison between sub-apical and basal as shown for the wildtype.

5. Is the difference between the CoV of ring size for elav>Abl; ablnull significantly different from LL54>Abl;ablnull? In the representative image it appears the elav rescue is not as "strong" for the basal ring size and there's more variance in ring and intensity at the basal side whereas the LL54 rescue visually appears more similar to wildtype. So, while expressing Abl in photoreceptors incredibly rescues the ablnull phenotype to some extent, it appears that the effect is stronger when Abl is expressed in the IOPC cells. I think it would be interesting for the authors to comment on the difference between these rescues, especially since I would have thought the opposite would be true since the photoreceptors are smaller and fewer than the IOCPC.

6. What is labeled in Figure 5I and J, F-actin and Ecad? If so, it looks like the Ecad organization in the elav>Abl rescue is distinct from that of the organization in wildtype and LL5>Abl. Where Ecad is more dispersed along the apical-basal axis of the IOCPC while in the wildtype and LL5>Abl Ecad is more restricted apically. Or perhaps this observation is just anecdotal to these representative examples. If it is not, it would be interesting for the authors to comment on how driving Abl expression in photoreceptors could influence the Ecad localization in IOCPC cells that are ablnull.

7. Figure 7 schematics are different than the previous schematics – could they be made to match the style of the schematics in Figure 1F and G? I found it difficult to understand which lines and shading were cells. For example in Figure 7A (top-left) I don't know which are the rhabdomeres, but in 7B (right, mutant descriptions) the dark green lines now indicate rhabdomeres.

Additionally, I would eliminate the shaded part of Figure 7B, unless the authors want to directly address the force patterns in the WT and different mutants (either by carefully dissecting and comparing cell shapes, or by tissue ablation).

Minor comments

1. For readers that are not familiar with the retinal epithelium, adding labels for the different cell types in the schematics in Figure 2 would be extremely helpful.

2. Figure 5 – label lateral (A-D), apical(E-H), basal (I-L) on figure. Maybe even add an Ecad label for I-L for added increased clarity.

3. Figure 5M,N, and 6G,H, K – write out genotype a bit more clearly. For example, elavAbl; abl should be elav>Abl; ablnull the shorthand is harder to understand and to match to the images and text.

4. I would suggest changing from red/green for two color images to magenta/green or a different LUT combination that is red-green color-blind accessible.

First revision

Author response to reviewers' comments

Point by Point Response to Reviews
Reviewers 2 and 3 found the work interesting and commented positively about the quality of the imaging and experiments. However, both agreed with Reviewer 1 that the results were at times overinterpreted and that the phenotypes should be described more clearly and rigorously. All three reviewers offered helpful comments and suggestions for addressing their concerns.

In response, and as described below, major revisions have been made. Most significant are the addition of new phenotypic analyses (Figs 1K, 1M, 1N, 1P, 2E, 2F, 5M, 5N, 5P) and controls (Figs 4G, S1A), relegation of the "3D scaffold" idea to the discussion and Fig 7 and refocusing the manuscript more clearly on the role of Abl in 3D organization of the retinal epithelium. These and other major changes are highlighted in yellow in the manuscript text.

Reviewer 1 raised one major point and made several additional comments. We address each below.

Major point: The reviewer comments that we don't explain what we think causes the photoreceptors to fall, and that this is critical to understanding why the partial rescue experiments not only restore pattern but also prevent the falling.

We agree with the reviewer that the axonal connections to the brain could provide such a downward pulling force, and that when ommatidial organization is disrupted by *abl* loss, the photoreceptors become susceptible to being dragged basally. We have added a paragraph at the end of the discussion in which we discuss the photoreceptor falling phenotype and explain how in the partial rescue experiments, restoration of either junctional adhesions within the photoreceptor clusters or retinal floor pattern could be sufficient to resist this downward pulling force. Additionally, as part of our effort to emphasize the role of Abl in the 3D organization of the epithelium as suggested by Reviewer 3, we have switched the order of Figures 1 and 2. In this way, the photoreceptor falling phenotype and loss of epithelial integrity are presented as consequences of the defects in photoreceptor and IOPC cellular morphogenesis, rather than as the motivating starting point as we had framed it in the original submission. We feel this restructuring better highlights the novel findings of our work (the interdependence of photoreceptor-IOPC morphogenesis and its importance to 3D retinal organization) and avoids over-emphasizing the falling phenotype.

Additional comments:
1. The reviewer commented that because *abl* null animals are pupal lethal, those that survive to 100% p.d. may have milder phenotypes, explaining the "improved" apical network pattern we had noted. We agree this is possible and have added the following: "Qualitatively, as seen in the representative images shown in Figure 1F-1H, tissue-level apical pattern appeared to improve over time, with the regular lattice-like gridwork of ommatidia more obvious at later stages than at 50% p.d.. Because we could not track and image individual pupae over the two-day developmental window, this apparent "improvement" could simply reflect a failure of *abl* null animals with more severe phenotypes to survive beyond 50% p.d.. Alternatively, continued optimization of cell-cell apical contacts during the elongation phase might enable modest recovery of pattern.
Live imaging over long time scales coupled with analysis of tissue-level apical pattern will be needed to explore this further.." We still include the alternate possibility of partial recovery of pattern for two reasons: 1) if the milder late stage apical defects simply stem from only the healthier animals surviving that long, then we would expect basal pattern (which we imaged in the same retinas) to show a similar trend – however we see basal pattern is more disrupted at later stages; and 2) because the photoreceptors fall from apical-to-basal, it seems to us logical that there might be a temporal progression in how the physical disruption of the falling process manifests at apical vs basal planes, and so perhaps once the disruption has passed, there might be some correction in the apical planes. We are keen to live image over sufficiently long time scales to capture these events and changes in 4D, but it is challenging and well beyond the scope of this study.

2. Although the reviewer appreciates the difficulty of live animal imaging deep into a tissue like the fly retina, they comment that the video of the *abl* null retina shows little interpretable change over the time course imaged, that the color overlays in Video S2 are problematic, and ask why the left-most falling photoreceptor is shown as coplanar with a green photoreceptor in the basal view

when the two do not appear to overlap in the lateral view.

First, it is important to remember that the morphogenetic events are unfolding over long multi-hour time scales and that our imaging was limited to 1.5hr. We acknowledge that this means that the changes captured will be modest. Despite this, we feel the videos and the selected stills in the figures provide a useful qualitative impression of the dynamic irregularity in cell shapes, positions and cell-cell contacts between photoreceptors and IOPCs upon *abl* loss. The still fixed images and reconstructions capture some of this, but we feel the videos significantly enhance appreciation of the 3D spatial relationships between these cell types. We also acknowledge that the color overlays were extremely challenging in the *abl* mutant where the cell-cell contacts that normally define cell shape and ommatidial pattern were greatly disrupted. However, we are confident that from the many repetitions we performed, that the data we show are representative and that the color overlays, despite their limitations, provide an accurate perspective for appreciating the tissue disorganization.

To address the comments, we have removed the statement that we can "detect appreciable change" from these movies, and revised the paragraph to emphasize the insight into the 3D spatial relationships they provide: "In contrast, the shapes, positions, shapes and contacts of abl^{null} retinal cells were aberrant and irregular (Figure 2L; Figure S2C and S2D). Photoreceptor cell bodies were found mispositioned basally at or below the plane of the IOPCs, confirming our previous observations (Xiong and Rebay, 2011), and the organized apical membrane bundles (rhabdomere precursors) seen in wildtype were not apparent (Figure 2K). Occasional photoreceptors appeared to have lost contact with surrounding retinal cells, with the bulk of their cell volume beneath the IOPC cell bodies (Figure 2L, cyan cells). IOPC cell shapes were also aberrant (Figure 2K and Video S2), disrupting the cell-cell contacts that pattern the apical and basal networks (Figure S2C and S2D)."

Regarding the confusion about the apparent inconsistency in lateral and basal views in Video 2, we have added the following explanation to the legend for Figure S2D: "If comparing the stills shown in **(D)** with Video S2, please note that the plane of sectioning for generating the lateral view video passes through the 4 labelled cells visible in the basal plane. Between ~75-85 min in the video, the most apical part (neck) of the 5th cell (the left-most cyan falling photoreceptor) is visible in the basal xy view, but not within the plane of sectioning and therefore is not visible in the lateral view. Similarly, the majority of the cellular volume seen in the lateral view falls below the left-most magenta cell and so is not seen in the xy basal view."

3. The reviewer commented that the 100% p.d. wildtype lateral reconstruction might be confusing because it does not include sections showing the retinal floor. Because we wanted this image (Figure 2C) to highlight the rhabdomere organization along the full longitudinal axis, and to give a bit of a 3D impression, and because we were already imaging 100µm into the tissue, we did not attempt to include the retinal floor. To address the comment, we have annotated with "retinal floor" as we do in the *abl* null retina (2D).

4. The reviewer asks for more description of what was formerly shown in Fig 4G,G'. Because this was a very tangential point, we opted to remove these data.

5. The reviewer asks how the "non-enrichment" of Ena in the 50% p.d. IOPC feet should be interpreted given there is very little F-actin accumulation at this stage. We agree with this point and have deleted the comment, and now point out that "At the basal plane, Ena was prominent in bristle cells".

6. Finally, the reviewer pointed out several apparent discrepancies between this work and our earlier characterization and interpretation of *abl* loss of function phenotypes (published in papers in 2011 and 2013). We appreciate these comments and have made multiple revisions to the text that refer to our previous work, pointing out both similarities and differences. We hope these changes have improved the clarity and accessibility of our results.

- Regarding Abl cellular localization, we have revised the text to acknowledge the conclusions of previous work, both ours and others: "Previous antibody-based analyses of Abl protein localization in the developing retina emphasized its enrichment in the photoreceptor apical

membranes (Bennett and Hoffmann, 1992a; Xiong and Rebay, 2011) although Bennett and Hoffmann also noted low levels of Abl protein within the IOPC apical network at 25% p.d.."

- Regarding the disorganization of the photoreceptor apical membranes: "consistent with our earlier study of the *abl* null phenotype (Xiong and Rebay, 2011), in *abl* mutant retinas, the photoreceptor apical domains were no longer aligned correctly along the longitudinal axis, their apical anchor points had dropped more basally and the adherens junction belt appeared reduced (Figure 2B, 2E and 2F)".
- Regarding the implied discrepancy that our wording suggested regarding whether or not *abl* mutant photoreceptors form rhabdomeres (the claim in Xiong and Rebay 2011 that they do not is correct) we have corrected the language "By 100% p.d., the well-aligned bundles of long rod-like rhabdomeres seen in wildtype (Figure 2C) were not detected in *abl* null ommatidia (Figure 2D).

Instead, a tangled mass of Ecad-marked apical membrane….".

- Regarding the dedifferentiation model we proposed in Xiong et al 2013, we now state clearly that the model was wrong: "These results confirmed our prior report of progressive photoreceptor "loss" from the retina but refuted the dedifferentiation mechanism we had proposed (Xiong et al., 2013). Instead, our analysis suggests that the cellular defects associated with Abl loss perturb the 3D organization and integrity of the epithelium, with photoreceptor "falling" a component of the cell and tissue scale disruptions."
- We agree our attempt at simplifying by referring to photoreceptor apical surfaces as rhabdomeres at all stages could be confusing, particularly in describing the *abl* mutant phenotype, and have deleted that statement. We now refer to them as photoreceptor apical membranes or rhabdomere precursors at 50% p.d. and as rhabdomeres at 75 and 100% p.d.
- Regarding the comment that our cartoon schematic of the *abl* 50% p.d. ommatidium is quite different to that shown in Xiong and Rebay 2011, we agree they are not identical but disagree the differences are substantial. Our intent was to update the cartoon, incorporating the better understanding of 3D cell shapes and positions provided by the current work. In 2011 we did not realize that the retinal floor was mispatterned and breached and so drew a simpler model focused on the relative mispositioning of the photoreceptor cell bodies (nuclei).
  We have adjusted the text to refer to the previous study: "Photoreceptor cell bodies were found mispositioned basally at or below the plane of the IOPCs, confirming our previous observations (Xiong and Rebay, 2011), and the organized apical membrane bundles (rhabdomere precursors) seen in wildtype were not apparent (Figure 2K). Occasional photoreceptors appeared to have lost contact with surrounding retinal cells, with the bulk of their cell volume beneath the IOPC cell bodies (Figure 2L, cyan cells)."

Reviewer 2 was overall very positive regarding the quality of the data and execution of experiments and found the main finding of interdependent cell morphogenesis revealed by the partial rescue experiments novel and of broad interest. The main criticisms were that more quantitative analysis of the phenotypes was needed and that some of the interpretations were unwarranted, particularly regarding tension inferences. The reviewer provided a very long and very helpful list of specific comments for how we might strengthen some of the key conclusions. We explain our response to each below.

1. We now mention the R8 nucleus is basal: "In wildtype, photoreceptor nuclei (with the exception of R8 cell nuclei that reside basally)…" and in the figure legend for 2K we now comment that we identify fallen *abl* mutant "fallen photoreceptors (cyan, identified by dense apical membranes and position)". At 50%, although the photoreceptor apical membrane bundles are disorganized (see Fig 2B) they are readily detected with the cellmask membrane label, which makes photoreceptor identification "easy" (once you become familiar with the tissue). We are completely confident in the cell type identification in the videos.

2. The reviewer asks for more quantitative description of rhabdomere organization/disorganization. We have added panels 2E and 2F showing 50% p.d. measurements of the distance from the apical surface of the epithelium to the apical tip of the photoreceptor apical membrane bundles and the angle from the vertical. We think these metrics capture the two most

important features of the phenotype: 1) the basal- ward collapse and 2) the loss of optical axis alignment. The 100% phenotype was too severe to measure anything other than retinal depth.

3. We have removed the overly interpreted conclusion at the end of the first section of the results and replaced it with: “Together, these observations suggest that Abl function is broadly required in multiple cell types to establish and maintain the 3D organization of the retinal epithelium.

4. We deleted the sentence “Scaffold alignment and connections are maintained throughout the elongation phase despite extensive remodeling of cellular structures”. Mention of the scaffold has been relegated exclusively to the discussion.

5. We deleted the comment about “uneven spacing between neighboring ommatidia” because we agree it is not easy to appreciate from the data shown.

6. The reviewer asks whether by including the rhabdomeres in the “scaffold” we are implying that their elongation is driving retinal elongation. No, that is not our intent. The intent of Figure 7 is to propose how the photoreceptor rhabdomeres, along with the IOPC apical and basal domains, provide a physically interconnected structurally supportive 3D network that channels forces productively across planes to coordinate the morphogenetic program. What actually triggers the initiation and continued elongation of the epithelium (and the rhabdomeres) is a fascinating question, but beyond the scope of our discussion.

7. As described in response to comments from reviewer 1, we have incorporated multiple changes to frame the current work in light of our earlier studies. We agree with the reviewer that the falling photoreceptors may act as cellular wrecking balls that further perturb cellular structures and connections as they collapse, and have included this in the final paragraph of the discussion: “Analogous to how oriented cell division and mitotic nuclear movements physically displace adjacent cells and redistribute patterns of mechanical tension and adhesion in simple epithelia to impact the morphogenetic program (Bosveld et al., 2016; Kondo and Hayashi, 2013; Leen et al., 2020; Mao et al., 2013), the collapsing photoreceptor clusters will disrupt cell shapes, contacts and force balances within each ommatidium, further perturbing pattern across all tissue planes.”

8. The reviewer comments that our 75% p.d. wildtype apical panel (Fig 1D) does not look wildtype, presumably because of mounting issues. The reviewer is correct, and probably also appreciates the difficulty of the 75% p.d. dissection relative to the other two time points we use in our experiments. The challenge was even greater because for each time point and genotype in Figure 1C-H”) we imaged the same retina at both apical and basal planes. So instead of trying to repeat the entire analysis, we instead now show an example of a fully wildtype looking 75% apical pattern in Fig S1A – by not also trying to show the basal planes, it made it easier to optimize the mounting to show apical.

9. We have removed all the tension inferences.

10. As explained above in response to comments from reviewer 1, we have removed the interpretation that “cone cells dropped sub-apically”.

11. The reviewer comments that the claim of improved apical network pattern by 100% p.d. in the *abl* null is difficult to appreciate in the images (Fig 1F-H) or in the measurements of 2^{o} IOPC apical length (Fig 1I). We added a sentence to help the reader appreciate the qualitative differences that led to the comment: “Qualitatively, as seen in the representative images shown in Figure 1F-1H, tissue-level apical pattern appeared to improve over time, with the regular lattice-like gridwork of ommatidia more obvious at later stages than at 50% p.d..” However, the reviewer is correct that our current measurements do not capture this change as significant (with the exception of the IOPC apical length where the decrease in variability between 50 and 100% is significant, but of course subject to the caveat pointed out by reviewer 1, and so is not pointed out). Because of the complexity of the phenotype, we think machine learning based approaches to analyze broader scale tissue pattern will be needed to determine definitely if there is apical network pattern improvement, but until we can follow changes in individual retinas over long time scales, we are reluctant to invest too much effort into this.

12. We removed the interpretation that ring size heterogeneity tells us anything about tension.

13. We added quantification of the basal F-actin stress fiber intensity in the IOPC feet at 75 and 100% p.d. (Fig 1M & 1N). The results are informative and we thank the reviewer for the excellent suggestion.

14. The reviewer asks about the timing of photoreceptor falling, concerned that if *abl* mutant photoreceptors end up trapped beneath the retinal floor during the patterning phase, then phenotypes characterized much later during the elongation phase would provide little insight into morphogenetic program per se. As characterized in Xiong et al 2013 and subsequently re-confirmed as part of this study (but not included in the paper) the progressive "loss" of photoreceptor cell bodies from the retinal epithelium begins at/after 50% pd., i.e. it all occurs during the elongation phase, not before.

15. We removed the vague term "rhabdomere organization" in describing Figure 4A-B" and replaced it with a more specific description of the phenotype : "in contrast to *abl* null ommatidia where only scattered foci of F-actin were observed (Figure 4A), organized bundles of rhabdomeric F-actin were seen when *ena* dose was reduced (Figure 4B). Similarly, Elav-positive photoreceptor nuclei were again detected at the appropriate plane upon *ena* reduction (Figure 4D vs. 4C). " We did not attempt quantitative analysis in the clones (as suggested by the reviewer) for two reasons. First, we use them as simple Yes/No readouts of a genetic suppression interaction – Are organized rhabdomeres detected? No in *abl* null; Yes upon reducing *ena* dose. Are photoreceptor nuclei found at the correct tissue plane? No in *abl* null; Yes upon reducing *ena* dose. Second, there is significant non-autonomy (consistent with the main finding of this paper of R-IOPC interactions) which complicates quantitative measurements in the clones – this is an assay we intend to explore and exploit in our future studies of the mechanisms of interaction, but at present it falls beyond the scope of this paper.

16. The data shown in what was formerly panel Fig4E have been removed.

17. The reviewer asks whether the faint basal signal detected with anti-Ena is background or real signal. Honestly, we aren't sure. In our hands the antibody is really pretty bad. We are confident in the apical pattern, but the antibody seems to penetrate the tissue very poorly, making it difficult to conclude anything more than there is enrichment in the bristles. Further as reviewer 1 notes, at 50% there is so little F-actin in the basal feet that one wouldn't expect much Ena enrichment. We tried to look at later stages but could not get the antibody to work. We have moved the 50% data to the supplement (Figure S4A-S4B) and have modified the text in the results: "We first confirmed Ena expression in both cell types. Ena protein was enriched in and overlapped with F-actin in the photoreceptor apical domains and was detected in the IOPC apical domains (Figure S4A and S4A'). At the basal plane, Ena was prominent in bristle cells, but little, if any, was detected in the IOPC feet, consistent with the minimal F-actin enrichment at this stage (Figure S4B and S4B').

18. The reviewer recommended additional controls and measurements to accompany the experiment in which we show that photoreceptor-specific knockdown of Ena, but not IOPC-specific knockdown, suppresses the *abl* null phenotype (now Figure 4E-G; formerly Figure 4H,I). We added what we think is the key control experiment as Figure 4G – this shows that IOPC-specific knockdown of Ena on its own does not have a phenotype that might mask a suppressive interaction: "Confirming that IOPC-specific Ena knockdown alone did not disrupt retinal pattern and thereby mask a suppressive interaction, control retinas appeared wildtype (Figure 4G, compare to wildtype in Figure 2A)." We did not explore Ena RNAi knockdown phenotype in the photoreceptors, as even if there are defects, it would only strengthen the suppressive interaction result seen in Fig 4E. We also did not perform the requested measurements as it was simply too huge of a task to redo the experiment on a sufficient scale for that – it is tricky enough to get *abl* null animals at the correct stages, but once we start adding additional genetic elements, the recovery drops further, and we set a very high bar for quality of data that we use for 3D reconstructions. However, generating quality, measurable apical plane only data is much easier, and so we have added panels H-L to Figure 4.
Overall we are confident that all images shown are representative, and we think the qualitative differences are obvious and speak for themselves. Finally, in Figure 4A-F we show Abl GFP expression driven either by elav or LL54 – these images were captured the same day using identical

confocal settings. Although we have not attempted a quantitative comparison of GFP levels (it is really non-trivial to do that rigorously), there do not seem to be obvious major differences in driver strength sufficient to skew our results.

19. We removed the interpretation of tension in the results describing Figure 5

Reviewer 3 found our findings and ideas exciting but felt that there was inadequate evidence for the proposed 3D cytoskeletal scaffold and suggested we either revise the manuscript to emphasize the role of Abl in 3D retinal organization or do more experiments aimed at defining the scaffold more mechanistically.

We have taken their advice and removed the concept of the 3D scaffold from the title and results. Replacement sentences that emphasize 3D organization are highlighted in the text. Given the reviewer found the idea exciting, we introduce it as a speculative model in the discussion and Figure 7.

Additional comments:

1. The reviewer suggests replacing the 3D rendered ommatidial schematic in Figure 1 with something more helpful to someone unfamiliar with the system. Rather than showing multiple stages, we opted to replace it with a more generic and more simple “elongation stage” ommatidium cartoon that emphasizes the two key cell types we study and that matches the design of schematics used in subsequent figures.

2. We removed the scaffold cartoon (former Figure 1F’) and the quoted sentence describing it. A new and simpler scaffold schematic is now shown in Figure 7B.

3. We now explain in both text and Figure 4 legend that Elav-Gal4 was used to drive photoreceptor-specific Ena knockdown and LL54>Gal IOPC-specific Ena knockdown: “To confirm the relevance of Abl-Ena antagonism in photoreceptors, we selectively expressed Ena dsRNA (Ena^{RNAi}) in the photoreceptors (using *elav-Gal4*) and as a control in IOPCs (using *LL54-Gal4*) in an abl^{null} background.”

4. We decided that showing Ena expression in an *abl* null was an unnecessary distraction to the story and removed it.

5. The reviewer correctly notes that qualitatively it appears that IOPC-specific Abl expression rescues “better” than photoreceptor-specific Abl expression and asks whether the CoV of ring size for elav>Abl; ablnull is significantly different from LL54>Abl;ablnull? It is not (it just misses the * significance cutoff). We were surprised by the observation as well – because the photoreceptors are specified and organized into clusters long before the IOPCs, we thought restoring Abl in photoreceptors would rescue more effectively. It is possible, as reviewer 2 points out, that differences in strengths of expression driven by elav vs LL54 account for this difference, but the genetic complexity of trying GMRgal4, elavgal80 in the *abl* null mutant has prevented us from testing this – we just don’t recover enough animals. We could of course do an extensive analysis of GFP levels using different cell type specific driver combinations, but the effort required doesn’t seem worthwhile, particularly if the genetics doesn’t make if feasible to actually test it in the *abl* experiments. We actually think the results is informative about the major contribution of the IOPCs to overall pattern and about the surprising “flexibility” of earlier defects to be corrected much later if the physical 3D environment is rescued.

6. Apologies for neglecting to label Figure 5I-L – we have corrected this. It shows Integrin.

7. We have changed the Figure 7 schematics so that they better match others in the earlier figures. The figure has also been simplified.

Minor comments:

1. As suggested, we have added cell type annotation to Figure 1C,1C’, 1D’ (formerly in Fig 2).

2. We have added the suggested labeling to Figure 5.

3. We have corrected the genetic notation as suggested in all figures (for example, elav>Abl; abl^{null})

Second decision letter

MS ID#: DEVELOP/2023/201757

MS TITLE: Inter-plane feedback coordinates cell morphogenesis and maintains tissue organization in the Drosophila pupal retina

AUTHORS: Xiao Sun, Jacob Decker, Nicelio Sanchez-Luege, and Ilaria Rebay

You will be pleased to know that the referees are happy with your revisions and there are just a couple of minor points to address before we proceed to publication. 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.

Reviewer 2

Advance summary and potential significance to field

This revised version reads well, and the quality of the data is excellent. The paper presents interesting work that will be of broad interest to the community of cell and developmental biologists interested in morphogenesis

Comments for the author

Minor comments:

- Pp12: We propose that the specialized …/… " This should be rephrased as "We speculate../…)" There is no direct experimental evidence for a mechanical coupling.

- Similar comment for "a 3D supracellular network". This is overinterpretation at this stage. What the experiments shows is that "cell types can coordinate their morphogenesis to maintain…/…"

- The authors aim to provide as many relevant references as possible to contextualise their work, which is commendable. However, I feel that some pertinent references are missing, and they might consider reviewing the paper for inclusion. For example, I suggest they mention the F-actin/bcatenin regulator Pak4 (Walther et al Cell report, 2016), and potential Abl interactor, in their list of factors required to photoreceptors to remain in the plane of the retina. Pp13" One appealing possibility is that photoreceptor axonal connections in the brain exert a pulling force (Langen et al., 2015)": Previous work from (Lee and Treisman, MBoC 2004) would seem relevant here.

Reviewer 3

Advance summary and potential significance to field

This revised manuscript by Sun and colleagues identifies a role for Abl in establishing and maintaining the three-dimensional (3D) stereotyped organization of the Drosophila pupal retina. They show that feedback between the different cell types can correct and rescue patterning defects in another cell type and vice versa.

The authors use careful 3D imaging of developing pupal retinas to characterize the effects of loss of abl on the retinal epithelium and its 3D organization. In this revised manuscript they move the idea of a "3D scaffold" to the discussion while making many helpful additions to the manuscript and figures to improve readability.

Comments for the author

The authors have made changes as requested. I have no further revisions to suggest.

Second revision

Author response to reviewers' comments

Response to reviewer 2.
1) As requested in the Discussion session, the verb "propose" was replaced with "speculate" and we replaced "3D supracellular network" with "how photoreceptors and IOPCs"
2) We now include both Rap1 and Pak4 in the list of photoreceptor falling mutants, with appropriate references
3) We added the suggested Lee & Treisman reference

Third decision letter

MS ID#: DEVELOP/2023/201757

MS TITLE: Inter-plane feedback coordinates cell morphogenesis and maintains tissue organization in the Drosophila pupal retina

AUTHORS: Xiao Sun, Jacob Decker, Nicelio Sanchez-Luege, and ILARIA REBAY
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.