Dishevelled controls bulk cadherin dynamics and the stability of individual cadherin clusters during convergent extension

John Wallingford and Robert Huebner

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

RE: Manuscript #E22-06-0194
TITLE: "PCP controls bulk cadherin dynamics and the stability of individual cadherin clusters during convergent extension"

Dear John,

I hope you're well. It seems way too long since Les Diablerets!

Many thanks for submitting your work for consideration by Molecular Biology of the Cell. Two reviewers have now seen your manuscript. They are remarkably consilient in their comments. Both find your work interesting - technically and scientifically - and both recommend publication after minor revisions that will not require re-review.

Reviewer 1 points out that you really only use the Xdd1 fragment approach to assess PCP signaling, and so you should consider either doing other experiments to examine other PCP components or retitling the manuscript. This seems sensible to me because of variability in subcellular phenotypes associated with perturbation of various other PCP components besides Disheveled, as pointed out by the reviewer and based on my own sense of the literature. This reviewer also asks for a bit more thinking about temporal/functional ordering of PCP and adhesion per se, which seems sensible.

Both reviewers were hoping for more explicit thinking about cadherin clusters (is there a relation between size and lifetime, etc.). This is a helpful request, I think, because this may cause you to focus your mechanistic thinking a bit more in discussing your results.

In your response letter please state how you have addressed all of the other specific comments on the text and figures, typos/extraneous text, and other minor concerns.

Thanks again for considering MBoC. We look forward to receiving your revised manuscript.

Best regards,
Jeff Hardin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Wallingford,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

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Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us immediately at mboc@ascb.org.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL):
Link Not Available

Authors of Articles and Brief Communications whose manuscripts have returned for minor revision ("revise only") are encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract.

Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

The paper shares interesting observations concerning cadherin clusters in the Xenopus chordamesoderm during convergent extension. It shows that cadherin-3 becomes enriched in shortening cell-cell junctions where it forms transient clusters in synchrony with actomyosin condensations. Disruption of Dvl function desynchronizes cadherin and actomyosin dynamics, reduces the number of clusters and shortens their lifespan. Several points should be addressed before publication.

1 Title: "PCP controls...": actually, only Xdd1 is tested. In view of the different effects of other PCP factors on cadherins discussed by the authors (last paragraph), the title may appropriately refer to Dishevelled, not PCP in general.

2 Figure 1A: should be "t-junction", not "v-junction" in right cell quadruplet.

3 The Xdd1 construct used to disrupt Dvl function leads to a loss of chordamesoderm cell polarization and cell intercalation. It should at least be discussed, if not experimentally explored, whether the effects on cadherin clusters are upstream of these previously described polarity defects or downstream consequences, e.g. consequences of a presumed loss of the distinction between v- and t-junctions.

4 Enrichment of cadherin at "shortening v-junctions": it may be pointed out that enrichment is preferentially in the shortest v-junctions (fig.1C,F) (already shortened ones, assuming that all v-junctions are shortening eventually).

5 The legend to Figure 1 does not fit the figure panels and the main text. Therefore, it is not clear what the significance values correspond to, etc.

6 Page 4 paragraph 3: "UTTravel@Anthony.Travel.com" may make more sense in a different context.

7 Figure 4C-E: the effect of Xdd1 on lifespan is significant but small (4E). Nevertheless, could it quantitatively explain the nearly two-fold difference in cluster number (4C)? Also, are cluster size and lifespan interdependent? Are small clusters short lived, larger ones more stable? Or do larger ones break up into smaller ones?

8 Page 6 Conclusion: Xdd1 affecting only dynamic coupling, not amplitude or frequency of oscillations: any suggestions how this could be achieved mechanistically?

Reviewer #2 (Remarks to the Author):

This interesting study analyses cadherin dynamics during convergent-extension of the mesoderm in the Xenopus gastrula. One first observation is that cadherin levels do not decrease, but rather increase, during shrinking of the original (v) cell-cell junction, contrary to what classically observed for CE in the Drosophila embryonic blastoderm, where cadherin is planar polarized. This is one more indication that CE of a mesenchymal tissue such as mesoderm involves distinct mechanisms than CE of epithelial monolayers. The second observation is along cadherin concentration in "puncta" ("clustering") is dynamic along the v junctions and occurs by oscillations that correlate with actin dynamics. Finally, inhibiting the PCP pathway leads to uncoupling of cadherin and actin oscillation, as well as to a reduction in the cadherin cluster size and their life span.
The study also introduces a simple and neat way to analyse cluster size and life span using kymographs.
Altogether this is a nice piece of work bringing interesting insights into cadherin dynamics during a morphogenetic process.
One criticism, though: One would wish some more integration of the different parameters examined in this study, in particular, the potential link between the coordination cadherin clusters/actin on one hand, and cadherin cluster size and life span on the

other, and the effect of PCP inhibition on these two aspects? At the least the authors should comment on this issue.

Reviewer #1 (Remarks to the Author):

The paper shares interesting observations concerning cadherin clusters in the Xenopus chordamesoderm during convergent extension. It shows that cadherin-3 becomes enriched in shortening cell-cell junctions where it forms transient clusters in synchrony with actomyosin condensations. Disruption of Dvl function desynchronizes cadherin and actomyosin dynamics, reduces the number of clusters and shortens their lifespan. Several points should be addressed before publication.

1 Title: "PCP controls...": actually, only Xdd1 is tested. In view of the different effects of other PCP factors on cadherins discussed by the authors (last paragraph), the title may appropriately refer to Dishevelled, not PCP in general.

We agree with the reviewer that the title was overly general and have changed the title. The new title is “Dishevelled controls bulk cadherin dynamics and the stability of individual cadherin clusters during convergent extension”.

2 Figure 1A: should be "t-junction", not "v-junction" in right cell quadruplet.

The mis-labeled figure has been corrected; we thank the reviewer for highlighting this error.

3 The Xdd1 construct used to disrupt Dvl function leads to a loss of chordamesoderm cell polarization and cell intercalation. It should at least be discussed, if not experimentally explored, whether the effects on cadherin clusters are upstream of these previously described polarity defects or downstream consequences, e.g. consequences of a presumed loss of the distinction between v- and t-junctions.

This is an excellent point and we have made major changes to the discussion to explain that our data does not distinguish if the clustering phenotype is upstream of polarity/intercalation defects or if it is downstream. We prefer a model where the clustering phenotype is a downstream effect; with the logic being that Xdd1 is altering the actomyosin cytoskeleton and the clustering phenotype is a response to changes in actomyosin dynamics. The discussion now states that we expect that the clustering phenotype is likely secondary to perturbation of the actomyosin cytoskeleton.

4 Enrichment of cadherin at "shortening v-junctions": it may be pointed out that enrichment is preferentially in the shortest v-junctions (fig.1C,F) (already shortened ones, assuming that all v-junctions are shortening eventually).

We agree this is an important distinction. The text that directly refers to fig.1C states that “Cdh3 is enriched at the shortest v-junctions”. We have changed the text referring to Fig.1F to clarify this point. The text referring to fig.1F now reads:

“Quantification of mean Cdh3-GFP intensity at shortening v-junctions showed a clear enrichment of Cdh3 during junction shortening, such that the shortest v-junctions had

the highest Cdh3 intensity (Fig.1F)."

5 The legend to Figure 1 does not fit the figure panels and the main text. Therefore, it is not clear what the significance values correspond to, etc.

The figure 1 legend has been updated to now match the figure panels and the main text. Thank you for noting this error.

6 Page 4 paragraph 3: "UTTravel@Anthony.Travel.com" may make more sense in a different context.

This error has been corrected. It is unclear how our travel agent's email was introduced into the manuscript.

7 Figure 4C-E: the effect of Xdd1 on lifespan is significant but small (4E). Nevertheless, could it quantitatively explain the nearly two-fold difference in cluster number (4C)? Also, are cluster size and lifespan interdependent? Are small clusters short lived, larger ones more stable? Or do larger ones break up into smaller ones?

In response to the first question, yes, is possible that the reduction in lifespan can account for the reduced cluster number but it is also possible that cluster formation is disrupted by the Xdd1 mutant. Indeed, the cis-mutant shows a reduction in cluster number with no shift in cluster lifespan so it seems there is a mechanism that can prevent cluster formation that is not associated with lifespan.

As to the second question, cluster size and lifespan are not independent. In fact, there is a tight correlation between cluster size and lifespan, and we have included a new figure (Fig.3F) to show this result.

8 Page 6 Conclusion: Xdd1 affecting only dynamic coupling, not amplitude or frequency of oscillations: any suggestions how this could be achieved mechanistically?

We have decided to withdraw our conclusion that that Xdd1 is not altering the amplitude or frequency of oscillations. Upon review of our data, we find that we do not have sufficient evidence to make this claim and have changed the above sentence to read:

"However, Xdd1 controlled the coupling of the actin and cadherin dynamics which is a new phenotype relating to oscillations."

Reviewer #2 (Remarks to the Author):

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mesenchymal tissue such as mesoderm involves distinct mechanisms than CE of epithelial monolayers. The second observation is along cadherin concentration in "puncta" ("clustering") is dynamic along the v junctions and occurs by oscillations that correlate with actin dynamics. Finally, inhibiting the PCP pathway leads to uncoupling of cadherin and actin oscillation, as well as to a reduction in the cadherin cluster size and their life span.
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We thank the reviewer for this critique and have added a paragraph to the discussion proposing our model for how Dishevelled is linked to actomyosin/cadherin dynamics and cadherin clustering. The updated discussion states that we believe that Dishevelled is modulating the actomyosin cytoskeleton and that perturbation of Dishevelled modifies the actomyosin cytoskeleton such that actin and cadherins are poorly coupled and cadherin clusters are destabilized. We hope the new discussion points satisfy the reviewer.

RE: Manuscript #E22-06-0194R
TITLE: "Dishevelled controls bulk cadherin dynamics and the stability of individual cadherin clusters during convergent extension"

Dear John,

Thanks for your patience while I was traveling. I am pleased to inform you that your manuscript is ready for publication in Molecular Biology of the Cell. I found a few minor typos that you should consider addressing (see below).

You may also wish to submit your kymograph cluster analysis methods as a Bio-Protocol or other publicly available protocol. Like one of the reviewers, I found that to be a creative and intuitive methodology that we may use in my own laboratory.

Thanks again for submitting your interesting work to MBoC!

Best regards,
Jeff Hardin
Monitoring Editor

(1) Highlights:
"Cadherin cluster lifespan and size are highly heterogenous." should be "...heterogeneous."

(2) Abstract:
"We then found that had cadherin and actin had coupled temporal dynamics and that disruption of planar cell polarity uncoupled these dynamics.": delete the first instance of "had".
"Next, using super-resolution time- lapse miscopy and quantitative image analysis we were able to measure the lifespan and size of individual cadherin clusters.": "miscopy" should be "microscopy"

(3) p. 3: "While PCP and cadherin-based cell adhesions have been studied to great depth" should be "...in great depth"
"Here, we show that Cdh3 was enriched specifically at shortening cell-cell junctions during Xenopus CE": should be "...is enriched..."
"These data not only improve our understanding of two essential molecular networks, PCP and cadherin-based adhesions, but also deepens our understanding of the molecular control" should be "...but also deepen our understanding..." ("data" is plural)

(4) p. 4: I think "immuno-staining" should be a single word.

Sincerely,
Jeff Hardin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Wallingford,

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In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised version, and figures, please use this link (please enable cookies, or cut and paste URL):
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Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

RE: Manuscript #E22-06-0194RR
TITLE: "Dishevelled controls bulk cadherin dynamics and the stability of individual cadherin clusters during convergent extension"

Dear John,

Thanks for sending in your final manuscript, which is headed for Production.
Thanks for choosing MBoC!

Best regards,
Jeff Hardin
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Wallingford:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
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