Alignment of the cell long axis by unidirectional tension acts cooperatively with Wnt signalling to establish planar cell polrity

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Original submission

First decision letter

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MS TITLE: Alignment of cell long axis by unidirectional tension acts cooperatively with Wnt signalling to establish PCP

AUTHORS: Sayuki Hirano, Yusuke Mii, Guillaume Charras, and Tatsuo Michiue

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development, provided that the referees' comments can be satisfactorily addressed. Both reviewers make very constructive comments. In my view, the suggestions from Rev 2 under their 1) point are not all required for publication. You may chose to address some of them if you have the data available. If you feel this would significantly delay publication, I suggest you discuss these issues for future work and simply address the other comments. Please detail how you address the comments from reviewers in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

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

Reviewer 1

Advance summary and potential significance to field

This paper explores the knotty issue of the long-range signals establishing PCP in epithelia, with a focus on morphogenesis in Xenopus. The paper is extremely complex, and is not helped by the

dense writing, but overall, the dataset will be extremely useful, and the findings are potentially very exciting. I recommend publication in Development.

The work is based on previous findings that gradients of wnt signals and/or gradients of mechanical tension can contribute to the establishment of PCP in epithelia. They extend these ideas into the morphogenetically active Xenopus posterior neural plate during early neurulation stages. They first define the onset of PCP in this tissue and use tissue-level laser cutting to show that tension along the AP axis is required for PCP establishment. Then, using tissue stretching experiments they show that tension is sufficient to set the PCP axis as has been shown for the Xenopus epidermis previously. They then use a clever combination of ectopic Wnt expression and Wnt inhibition to show that tension and Wnt signaling act additively to direct PCP. In the most interesting finds the paper ends with a detailed analysis of the basis of tension regulation of PCP, with their experiments arguing against a direct sensing of tension or of cytoskeletal action, and instead conclude that cell shape (elongation) is the key factor that establishes PCP.

Comments for the author

There is a huge amount of data here, and while not entirely conclusive on this final point, the result of the many, many experiments performed are most consistent with this novel and exciting hypothesis. One could of course conceive of innumerable additional experiments to demand of the authors, but this is the kind of thing that -in my view- is sucking the joy out of science.
I won't do it. I very much like the paper and I think the data are solid and the implications exciting. I strongly support publication. I will make only minor comments.

1. The data in Figure 1 must be quantified. It is 2021; pictures aren't enough anymore. Some higher magnification views of the data would also help.
2. I found it very hard to understand the "mean polarity value" used in several figures. This should be better explained.
3. The literature is vast, so authors can be excused for not knowing it all, but just the same there are several papers that scholarship demands the authors read and consider and ideally discuss in the present work.
Two papers from Beloussov are relevant to tension, cell shape and morphogenesis in Xenopus:
https://pubmed.ncbi.nlm.nih.gov/30273637/
https://pubmed.ncbi.nlm.nih.gov/10664152/
Two papers regarding microtubules, cell polarity, and Xenopus convergent extension bear mentioning:
https://pubmed.ncbi.nlm.nih.gov/9043070/
https://pubmed.ncbi.nlm.nih.gov/18270587/

Reviewer 2

Advance summary and potential significance to field

The study provides experimental support for a role of mechanical tissue tension in the establishment of PCP within the posterior neurectoderm of Xenopus.
Mechanical tension appears to act in concert with Wnt signaling gradients and affect PCP by elongating cells along the main tension axis.

Comments for the author

The ms is very well written and contains a number of potentially interesting observations. However, there are several points of criticism that need to be addressed before the paper is suitable for publication.
1. The main question arising from the data presented is how cell shape changes translate into polarized distribution of PCP components. Do any cytoskeletal elements display polarized distribution depending on cell shape? Is membrane-curvature related to PCP? Does the actomyosin cortex show an asymmetries depending on cell shape? Is the turnover rate of membrane/cortex bound proteins dependent on cell shape? The authors should come up with some explanation of how cell shape affects PCP.

2. The experiments that cell shape rather than tension controls cell shape are not entirely convincing. Could the authors perform an experiments where tension is applied to already elongated cells so that these cells would round up in response to anisotropic tension? If cells would not show PCP in such circumstances, then this would clearly show that shape rather than tension is controlling PCP.
3. For the experiments where tissue ablation is used to release tissue tension the authors should show that tissue tension does indeed relax.
4. The relationship between tissue tension and Wnt signaling needs some more quantitative assessment: do these two factors influence each other? Can Wnt signalling override tension anisotropy depending on the ratio of these two factors? Would an exogenous Wnt gradient opposite to the endogenous gradient still cooperate with the main axis of tension in controlling PCP?
Collectively, this is an interesting paper that should be published once revised along the lines suggested above.

First revision

Author response to reviewers' comments

Point-by-point responses to the comments

Let us first thank the reviewers for their many constructive comments. We believe that our manuscript has been significantly improved by addressing their suggestions as best we can. Below are detailed responses to each of them.

- *Reviewer 1 Comments for the Author:*
- *1. The data in Figure 1 must be quantified. It is 2021; pictures aren't enough anymore. Some higher magnification views of the data would also help.*

We have quantified the data in Figure 1 and confirmed that Vangl2 showed clear anterior-posterior (AP) polarization from stage 13. We have added the data to Figure 1 and revised the manuscript (lines 94-96).

- *2. I found it very hard to understand the "mean polarity value" used in several figures. This should be better explained.*

We have added explanations on the mean polarity value to the Results and the Methods sections (lines 133-136 and 456-468).

- *3. The literature is vast, so authors can be excused for not knowing it all, but just the same there are several papers that scholarship demands the authors read and consider and ideally discuss in the present work.*
- *Two papers from Beloussov are relevant to tension, cell shape and morphogenesis in Xenopus:*
  https://pubmed.ncbi.nlm.nih.gov/30273637/
  https://pubmed.ncbi.nlm.nih.gov/10664152/
- *Two papers regarding microtubules, cell polarity, and Xenopus convergent extension bear mentioning:*
  https://pubmed.ncbi.nlm.nih.gov/9043070/
  https://pubmed.ncbi.nlm.nih.gov/18270587/

We thank the reviewer for pointing out the papers that should be considered.

The first two papers are about how tension and cell movement influence to each other in controlling morphogenesis in *Xenopus* embryo. Although these studies are important, we did not add them into the reference list as they are not directly relevant to our present work on PCP regulation by mechanical signal.

The latter two papers show that microtubule polymerization is involved in planar polarity formation and associated convergent extension, although they focused on the mesoderm rather than the neuroectoderm on which we have focused. We considered that these papers were closely

related and important to our work and have added them into the reference list and revised the manuscript (lines 297-302).

- *Reviewer 2 Comments for the Author:*
- *1. The main question arising from the data presented is how cell shape changes translate into polarized distribution of PCP components. Do any cytoskeletal elements display polarized distribution depending on cell shape? Is membrane-curvature related to PCP? Does the actomyosin cortex show an asymmetries depending on cell shape? Is the turnover rate of membrane/cortex bound proteins dependent on cell shape? The authors should come up with some explanation of how cell shape affects PCP.*

The questions raised by the reviewer are interesting and worth examining, but it would take quite a long time to answer them. Therefore, we would like to leave them for future work, following the editor's suggestion. We have mentioned some possibilities for how cell shape changes translate into polarity in the Discussion section (lines 386-399).

- *2. The experiments that cell shape rather than tension controls cell shape are not entirely convincing. Could the authors perform an experiments where tension is applied to already elongated cells so that these cells would round up in response to anisotropic tension? If cells would not show PCP in such circumstances, then this would clearly show that shape rather than tension is controlling PCP.*

The suggested experiment would require a biaxial stretching device, which is unfortunately unavailable in our environment. Then, we conducted an alternative experiment to show that cell shape rather than tension is important for PCP control. That is, relaxing a pre-stretched chamber to induce cell shape change (See below). This experiment showed that the cellular major axis was aligned orthogonally to the relaxation axis, and that the orientation of the polarity axis changed into the same direction as that of the cellular major axis even when no exogenous tension was applied (See rose diagrams below). We have added these data to Figures 7 and S7 and revised the manuscript (lines 354- 364).

NOTE: We have removed unpublished data that had been provided for the referees in confidence.

- *3. For the experiments where tissue ablation is used to release tissue tension, the authors should show that tissue tension does indeed relax.*

Laser ablation method has been widely used to release tension. To confirm that tension was indeed released in our experiments, we examined cell shape change before/after ablation. When the tissue was cut perpendicular to the AP axis, the aspect ratio of the cells significantly decreased in anterior and posterior sides of the cut (below, left), while the aspect ratio of the cells on left and right sides of the cut did not change significantly when the tissue was cut parallel to the AP axis (below, right). These results show that the endogenous tension along the AP axis was released by laser ablation perpendicular to the AP axis. We have added these data to Figure S1 and revised the manuscript (lines 114-118).

NOTE: We have removed unpublished data that had been provided for the referees in confidence.

- *4. The relationship between tissue tension and Wnt signaling needs some more quantitative assessment: do these two factors influence each other?*

Honestly, we do not know at the moment whether the two factors influence each other. We think this is an exciting question to be elucidated in future work and have mentioned it in the Discussion section (lines 417-418).

- *Can Wnt signalling override tension anisotropy depending on the ratio of these two factors?*

To address this question, we did an extra stretching experiment. Wnt11 was overexpressed on one side of explants with three types of doses (0 pg, 300 pg or 600 pg), and all the explants were stretched in the AP direction at the same stretching rate (See below). When no mRNA for Wnt11 was injected, the accumulation of Prickle3 was in the AP direction, following the direction of stretch. On the other hand, when 300 or 600 pg of *Wnt11* mRNA was injected, the accumulation of Prickle3 was in an intermediate direction between the direction of stretch and that of Wnt gradient. We did not find any difference in the direction of Prickle3 accumulation between 300 pg and 600 pg of *Wnt11* mRNA. This result suggests that increasing the amount of Wnt ligand is not capable of overriding the effect of tissue stretch. We have added these data to Figure S4 and revised the manuscript (lines 229-231).

NOTE: We have removed unpublished data that had been provided for the referees in confidence.

- *Would an exogenous Wnt gradient opposite to the endogenous gradient still cooperate with the main axis of tension in controlling PCP?*

Yes. We have showed in Figure 4E-4H that exogenous ML-directed Wnt gradient (= orthogonal to the endogenous AP-directed Wnt gradient) cooperated with ML-directed tissue tension to establish PCP in the ML direction.

Second decision letter

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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 2

Advance summary and potential significance to field

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Comments for the author

The ms has been revised along the lines suggested by the two reviewers. Overall the ms has considerably improved. The only remaining concern I have is about the clear distinction between shape and tension in orienting PCP. The experiment with relaxing tension is not really very clear as compressive forces might orient PCP rather then the observed shape changes. Perhaps the authors could identify a few more elongated cells in their tissue (I assume there will always be a few such cells), exert tension perpendicular to the elongation axis of these cells and then check whether this leads to reorientation of PCP. This experiment should be possible with the device available.