Capillarity and active cell movement at mesendoderm translocation in the Xenopus gastrula

Martina Nagel, Debanjan Barua, Erich W. Damm, Jubin Kashef, Ralf Hofmann, Alexey Ershov, Angelica Cecilia, Julian Moosmann, Tilo Baumbach and Rudolf Winklbauer
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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 raised some concerns and recommend a substantial revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, 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' concerns.

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

This is an interesting paper proposing that the behavior of the LEM tissue during Xenopus laevis gastrulation can be explained by its liquid-like properties and the interaction with its overlaying ectoderm-derived BCR. In addition to the detailed morphological analyses of LEM cells by EM and X-ray phase-contrast micro-tomography (TXPCμT), at the cell and tissue levels, the authors addressed possible molecular interactions between LEM cells and BCR surface as well as the gap between them and demonstrated that PDGF-A signaling likely to be mediated by KDPak-1 can induce lamellipodia, which strengthened their hypothesis. Finally, they propose that the mechanism may be conserved among the primitive vertebrate species. Overall, this paper represents a comprehensive set of well-performed observations and analyses with scientifically sound methodologies. Especially interpretation of cell and tissue dynamics during morphogenesis from the viewpoint of physics would facilitate the understanding of tissue mechanics early development and organogenesis, and this work has a potential to contribute to further advancement of the field.

Comments for the author

However, the following major issues limit the impact of the study.

Major points

1. It seems that the attachment of tip cells to BCR and their oriented cell movements is critical to temporarily detach the LEM cells from BCR and maintain the gap where PDGFsf emanated from BCR is included. The idea is supported by the embryo morphology (a bulge/cusp of BCR wall at the attachment site) by EM and TXPCμT. However, there is no experimental data to support that the tip cell is actually pulling BCR inward except that the authors predicted that from the tension diagram (Fig.7, D, E). In order to discuss tissue mechanics from a physics point of view, supporting evidence is necessary. This could be tested by estimating residual stresses from tissue (BCR) deformation just after cutting BCR animal side of the tip cells of a live embryo, rather than removing whole BCR after fixation. Similarly, the discussion on the physical role of the LEM cells' shingle cell arrangement and the animally-oriented movement against the BCR epiboly, is interesting and colorable but also not well supported by experimental data, especially of tissue mechanics. In addition to the correlation between the extent of LEM cells-BCR contact and LEM cells' velocity of movement, the proof for the friction force which may resides in between the two tissues would be necessary.

2. All the data in the present work and previous reports seem to suggest that Pak-1 acts downstream of PDGF if it executes indirectly. However, data to show their epistatic relationship are weak. For example, can the loss of lamellipodia by PDGF-depletion rescued by KDPak1? At least, the authors need to elaborate more on the possible cross-talk of the two signaling components.

Minor points

1. For quantitative and statistical analyses, it is important to define cell protrusions "lamellipodia" and "filopodia" in more detail. What are the morphological criteria (width/length?) to count and distinguish them as distinct structures? A brief description in Materials and Methods as to how they are counted is helpful.

2. P5, Line 1, "Marginal lamellipodia are still absent (Fig. 2C)." does not make sense as the photo seems to show marginal lamellipodia (white arrows). Correctly, Fig. 2C may be Fig. 2B, or correct sentence may be "On FN coated substratum, lamellipodia extend freely from the margin" the cells are cultured on FN (Fig. 2C)."as in the legend.

3. P5, spell-out csFN, Fig. 3, legend, PDGFsf (short form?) and PDGFlf (long form?) and useful to describe their properties (diffusible and non-diffusible, respectively?) in the text at first appearance.

4. In Fig 3, E, membrane structures are all counted simply as lamellipodia despite that FNMO+KDPak1-induced “filiform” (panel A) and CadMO+KDPak1-induced “lamellipodia” (panel B) look very different. More precise classification of protrusions would be necessary. Alternatively, the authors need to show that the filiform indeed represent a kind of lamellipodia. This applies also to panels C and D in which filiform-like structures are observed.

5. Labelling of panels of Fig. 4 is inconsistent and confusing. The main text implies that all the specimens are “after removal of the BCR” but only C is indicated as “BCR removed”. In addition, Ccad “AB” and FN “AB” are labelled only for C series but not for panels A and B which are just indicated by colored letters Ccad (green)and FN (red). As all these are all immunostainings, these labeling should be unified for easy understanding. Alternatively the authors could put the labels in vertical direction on the left side of the corresponding panels.

6. P6, line 24, Fig 4G does not exist; it must be Fig 4F.

7. In Fig 4F and Fig S3, the cell body is obscure and should be indicated by a dashed line.

Reviewer 2

Advance summary and potential significance to field

This manuscript by Winklbauer and colleagues reports a beautiful study about amphibian mesoderm gastrulation, focusing on the leading edge of the involuting mesoderm (or rather endomesoderm). This study is remarkable in multiple aspects:
1) Compared to other morphogenetic processes, mesoderm migration is a topic has been left quite behind in most of the embryo models, partly because, even in models were particularly well suited for live microscopy such as zebrafish or Drosophila, the deep position of the internalized mesoderm is a serious limiting factor, that adds to the difficulty to specifically manipulate relevant cellular properties and dissect the contribution of intrinsic and extrinsic factors. Nagel et al circumvent this problem by a smart combination of examination of fixed samples, including at the high resolution of electron microscopy, experiments on explanted tissues, and X-ray tomography, which have been recently applied by Moosmann and colleagues to obtained extraordinary movies of live amphibians at a single cell resolution (and even below, see Figures 6-8).
2) This study addresses the fundamental question of the role of the leading edge in the collective migration of cell mass moving on a substrate. This question has been mostly addressed in vitro systems, using artificial substrates that are quite remove from what happens in living organisms. Thus, the great interest of this embryonic system with a physiological substrate (a cellular substrate, the ectoderm), which furthermore is itself undergoing a morphogenetic process (epiboly). The authors show how the small area of front lamellipodia play a crucial role in allowing and controlling this migration.
3) The authors highlight the biophysical challenge of this migrating tissue to prevent the adverse effects of retraction due to tissue surface tension, and of capillarity, a problem of broad interest both for morphogenesis and for tissue biophysics in general.
4) The data presented here show with unprecedented detail how these challenges have been solved in embryonic development. The solution includes, in addition to the importance of an intimate contact of the leading lamellipodia with the substrate, also the role of attractive cues emanating from the cell substrate (PDGF) to bias cell migration. Thanks to this bias, constant cell intercalation keeps the proper configuration of the leading edge required to prevent its collapse. The model that stems from these data shows once more how nature finds elegant solutions, at the same time highly sophisticated at the molecular level, yet surprisingly simple in terms of biophysical principles.
5) This manuscript is densely filled with all sorts of invaluable information, no doubt that it will inspire developmental biologists working on a variety of models for many years to come.

Comments for the author

I have only minute comments:
End of page 5: “Lamellipodia resemble those of 6 Pak1-inhibited cells when C-cadherin is knocked down alone (Fig.3B), but those of csFN morphants when both cadherins or C-cadherin and csFN are co-inhibited (Fig.3C,D; Fig.S1D).”

The sentence is a little obscure, something is probably missing.

Page 6: “csFN does not co-localize with the receptor integrinβ1”. It may be safer to state that csFN does not co-localize with bright integrin spots, as one should not exclude that it interacts with a more diffuse integrin pool. This may then account either for csFN being moved to lamellipodia, and/or for signalling at non-adhesive sites.

Also page 6: “Adhesion to C-cadherin in vitro is sufficient to induce lamellipodia in LEM cells (Fig.S3)” This should be Fig.S4

Just below: Fig.4G does not exist, it’s probably Fig.4F

Page 7: “Indeed, when PDGF-A expressing aggregates are placed on BSA under a coverslip, large KD-Pak1-type lamellipodia form (Fig.5D,E) at numbers resembling those in the embryo (Fig.1N), and retraction fibers detach smoothly (Fig.5D,E).”
Somewhat hard to find the verb, I suggest to move the ref “(Fig.5D,E)”:
“Indeed, when PDGF-A expressing aggregates are placed on BSA under a coverslip (Fig.5D,E), large KD-Pak1-type lamellipodia form at numbers resembling those in the embryo (Fig.1N), and retraction fibers detach smoothly (Fig.5D,E).”

Fig7D: The colours of the arrows and sy

First revision

Author response to reviewers' comments

We thank the reviewers for the very kind response to our manuscript and their constructive criticisms. We revised the text and figures and added new data as detailed below.

Reviewer 1 *Comments for the Author:*
However, the following major issues limit the impact of the study.

Major points
1. It seems that the attachment of tip cells to BCR and their oriented cell movements is critical to temporarily detach the LEM cells from BCR and maintain the gap where PDGFsf emanated from BCR is included. The idea is supported by the embryo morphology (a bulge/cusp of BCR wall at the attachment site) by EM and TXPC□T. However, there is no experimental data to support that the tip cell is actually pulling BCR inward except that the authors predicted that from the tension diagram (Fig.7, D, E). In order to discuss tissue mechanics from a physics point of view, supporting evidence is necessary. This could be tested by estimating residual stresses from tissue (BCR) deformation just after cutting BCR animal side of the tip cells of a live embryo, rather than removing whole BCR after fixation.

We added data to show that the LEM tip pulls at the BCR, resulting in a cusp. Since in our experience, cutting the BCR leads to complex responses, most of them unrelated to cusp formation (e.g. Luu et al. 2011), we document instead cases where tip cells momentarily detach from the BCR. This leads to a clean, sudden retraction of the BCR cusp (new Fig.S7E,F). The LEM tip cell rim remains largely in place, since tip cells on either side of the detached one remain attached. However, the rim collapses immediately when the BCR is removed in the living embryo (Fig.9A,B) which is pointed out now in this context (p.10 top). Together, this indicates tension on both sides of the tip cell-BCR contact. We also show additional dnPDGF SEM images (Fig.S8C,D) and mention in the legend that in 10 out of 11 cases no cusp develops when LEM movement is thus impaired (in one case, shown in Fig.9H, a slight indication of a cusp is visible).

Similarly, the discussion on the physical role of the LEM cells' shingle cell arrangement and the animally-oriented movement against the BCR epiboly, is interesting and colorable but also not well supported by experimental data, especially of tissue mechanics. In addition to the correlation between the extent of LEM cells-BCR contact and LEM cells' velocity of movement, the proof for the friction force which may resides in between the two tissues would be necessary.

The Heisenberg lab has described friction between advancing anterior mesoderm and epibolic ectoderm in the transparent zebrafish gastrula (Smutny et al. 2017), i.e. in a situation which is homologous to the Xenopus LEM-BCR configuration but optically more accessible. To reproduce their results for Xenopus, using X-ray tomography to visualize the movements of both layers under different conditions, is presently beyond our means but also beyond the scope of our paper. Instead, we argue that normal changes in LEM-BCR contact (attachment-detachment phases) and relative tissue velocity (diminishing speed of epiboly over developmental time) give *the same effects as corresponding changes induced experimentally in the zebrafish system. We refer to the Smutny et al. paper in the paragraphs where we interpret our results along these lines (p.10, 2nd and 3rd paragraph). In addition, we show a TEM image of close contact between BCR and LEM rear cells when the two tissues are attached (Fig.S7C). We realized that this cannot be seen from the SEM or X-ray images shown before.*

2. All the data in the present work and previous reports seem to suggest that Pak-1 acts downstream of PDGF if it executes indirectly. However, data to show their epistatic relationship are weak. For example, can the loss of lamellipodia by PDGF-depletion rescued by KDPak1? At least, the authors need to elaborate more on the possible cross-talk of the two signaling components.

In our previous paper (Nagel and Winklbauer, 2018) we placed both Pak1 and PDGF-A in parallel upstream of integrin_alpha5_beta1, which in turn controls lamellipodia. In the present paper, where we cite Nagel and Winklbauer, 2018, we use KD-Pak1 only as a convenient tool to control lamellipodia formation, and do not attempt to examine epistatic relationships with PDGF-A. No respective experiments are included here, and due to space limitations, we did not elaborate in the abstract on the cross talk between the two components.

Minor points
1. For quantitative and statistical analyses, it is important to define cell protrusions "lamellipodia" and "filopodia" in more detail. What are the morphological criteria (width/length?) to count and distinguish them as distinct structures? A brief description in Materials and Methods as to how they are counted is helpful.
We added a clarification to the legend to Figure 1.

2. P5, Line 1, "Marginal lamellipodia are still absent (Fig. 2C)." does not make sense as the photo seems to show marginal lamellipodia (white arrows). Correctly, Fig. 2C may be Fig. 2B, or correct sentence may be "On FN coated substratum, lamellipodia extend freely from the margin" the cells are cultured on FN (Fig. 2C)."as in the legend.
We corrected this error.

3. P5, spell-out csFN, Fig. 3, legend, PDGFsf (short form?) and PDGFlf (long form?) and useful to describe their properties (diffusible and non-diffusible, respectively?) in the text at first appearance.

Done.

4. In Fig 3, E, membrane structures are all counted simply as lamellipodia despite that FNMO+KDPak1-induced "filiform" (panel A) and CadMO+KDPak1-induced "lamellipodia" (panel B) look very different. More precise classification of protrusions would be necessary.

Alternatively, the authors need to show that the filiform indeed represent a kind of lamellipodia. This applies also to panels C and D in which filiform-like structures are observed.
The origin of filiform processes in the collapse of lamellipodia is described in the text (p.5 section "csFN and cadherin are required…."), as is the difference between lamellipodia types.

We did not notice filopodia extending directly from the cell bodies.

5. Labelling of panels of Fig. 4 is inconsistent and confusing. The main text implies that all the specimens are "after removal of the BCR" but only C is indicated as "BCR removed". In addition, Ccad "AB" and FN "AB" are labelled only for C series but not for panels A and B which are just indicated by colored letters Ccad (green)and FN (red). As all these are all immunostainings, these labeling should be unified for easy understanding. Alternatively, the authors could put the labels in vertical direction on the left side of the corresponding panels.
Labelling has been unified, and "after removal of the BCR" has been removed since this holds for all panels in this figure.

6. P6, line 24, Fig 4G does not exist; it must be Fig 4F.
Corrected.

7. In Fig 4F and Fig S3, the cell body is obscure and should be indicated by a dashed line.
Dashed lines were added to indicate cell bodies in both figures.

***Reviewer 2** Comments for the Author: I have only minute comments:*

End of page 5: "Lamellipodia resemble those of 6 Pak1-inhibited cells when C-cadherin is knocked down alone (Fig.3B), but those of csFN morphants when both cadherins or C-cadherin and csFN are co-inhibited (Fig.3C,D; Fig.S1D)."
The sentence is a little obscure, something is probably missing.
The sentence was re-formulated as "Lamellipodia resemble those of Pak1-inhibited cells when C-cadherin is knocked down alone (Fig.3B). However, when both cadherins are co-inhibited, or C-cadherin together with csFN, they resemble those of csFN morphants, and retraction fibers persist (Fig.3C,D; Fig.S1D)."

Page 6: "csFN does not co-localize with the receptor integrinβ1". It may be safer to state that csFN does not co-localize with bright integrin spots, as one should not exclude that it interacts with a more diffuse integrin pool. This may then account either for csFN being moved to lamellipodia, and/or for signalling at non-adhesive sites.
The possibility of interaction with diffuse integrin, and putative consequences, are now mentioned.

Also page 6: "Adhesion to C-cadherin in vitro is sufficient to induce lamellipodia in LEM cells (Fig.S3)" This should be Fig.S4
This error was corrected.

Just below: Fig.4G does not exist, it's probably Fig.4F
Was likewise corrected.

Page 7: "Indeed, when PDGF-A expressing aggregates are placed on BSA under a coverslip, large KD-Pak1-type lamellipodia form (Fig.5D,E) at numbers resembling those in the embryo (Fig.1N), and retraction fibers detach smoothly (Fig.5D,E)."
Somewhat hard to find the verb, I suggest to move the ref "(Fig.5D,E)":
"Indeed, when PDGF-A expressing aggregates are placed on BSA under a coverslip (Fig.5D,E), large KD-Pak1-type lamellipodia form at numbers resembling those in the embryo (Fig.1N), and retraction fibers detach smoothly (Fig.5D,E)."
The sentence was changed as suggested.

Fig7D: The colours of the arrows and sy ??
Symbols were underlain to increase their visibility.

Second decision letter

MS ID#: DEVELOP/2020/198960

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AUTHORS: Martina Nagel, Debanjan Barua, Erich W Damm, Jubin Kashef, Ralf Hofmann, Alexey Ershov, Angelica Cecilia, Julian Moosmann, Tilo Baumbach, and Rudolf Winklbauer
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. Please also see Editor's note.

Editor's note:

The response to reviewer 2 and the corresponding revisions are appropriate.

Reviewer 1

Advance summary and potential significance to field

Although there are many literatures that describe functions of genes/proteins in morphogenesis of early embryos, the works that link cell/tissue mechanics with morphogenesis are only gradually increasing. This paper that combines state of the art microscopy technologies and molecular cellular biology represent one of such works and important in the developmental biology field.

Comments for the author

All the points raised by this reviewer have been adequately addressed and responded by the authors. This paper is now acceptable for publication.