Lmo7 recruits myosin II heavy chain to regulate actomyosin contractility and apical domain size in *Xenopus* ectoderm

Miho Matsuda, Chih-Wen Chu and Sergei Y. Sokol
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MS TITLE: Lmo7 recruits myosin II heavy chain to induce apical constriction in Xenopus ectoderm

AUTHORS: Miho Matsuda, Sergei Y Sokol, and Chih-Wen Chu

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

As you will see, the referees express considerable interest in your work, but have some criticisms and suggestions for improvement to your manuscript. If you are able to revise the manuscript along the lines 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.

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

The current study describes the LMO7 function in the apical constriction of ectodermal cells in xenopus embryos. Here, LMO7 is shown to bind Non-Muscle Myosin II A (NMIIA) through DUF4757 domain, and that LMO7 expression is required to localise NMIIA to the apical junctions. The role of LMO7 in apical size is further supported by loss-of-function experiments that contribute to the enlargement of the apical end-feet. As a read out of tissue tensions, the current study characterises the Wtip punctate and linear patterns at apical junctions following LMO7 gain-of-function. The role of LMO7 on apical constriction has been previously shown in Drosophila (Beati et al, 2018) but the current work potentially highlights the evolutionary conserved role of LMO7 between distinct animal species. Does LMO7 loss of function and apical constriction defects impairs neural tube/plate formation or morphology? answering this question would increase the novelty of the current findings.

Comments for the author

Points to consider:
1. Explain the changes in pigmentation in xenopus embryos.
2. the field commonly uses the apical end-feet size to infer apical constriction but other methods such as laser ablation and FRET should be complementary used.
The small size of apical end-feet does not necessarily indicate apical constriction. For example, nuclei position during interkinetic nuclear migration also affects the size of the apical end-feet.
3. Wtip dotted / linear pattern can be a good read out for tension, but this should be demonstrated in the current study by other methods such as laser ablation. For example, when LMO7 is overexpressed and a laser ablation is performed, would this transform Wtip linear pattern into a punctate pattern?
4. the quantification and statistical section only mentions the analysis of fluorescence intensity. It lacks information regarding the type of test used in other experiments such as apical end-feet size, or whether it is paired or an unpaired t-test, etc.

Reviewer 2

Advance summary and potential significance to field

The manuscript by Matsuda et al. describes functional and mechanistical studies of Lmo7 in regulating peri-junctional actomyosin bundles in Xenopus embryonic ectodermal cells. The authors show that Lmo7 is localized near the junctions of adjacent cells and can induce ectodermal cell apical constriction when ectopically expressed at high doses. Domain analysis reveals that DUF4757 and alpha-actinin binding motif are both required for induction of apical constriction. Lmo7 binds and recruits NMIIA to cell junctions to promote junctional actomyosin bundles. The authors conclude that Lmo7 triggers apical constriction by promoting NMII incorporation into peri-junctional actomyosin networks.

There are several new discoveries from the study, including the detection of Lmo7 protein localization (near cell junctions), the identification of a novel activity of Lmo7 (apical constriction), and the establishment of DUF4757-dependent interaction with and recruitment of NMIIA to the junctional actomyosin network. Despite these interesting results, several points need to be clarified, as indicated below.

Comments for the author

The Lmo7 protein localization is shown only in the enface view. A side view of Lmo7 localization with ZO1 (or cadherin), F-actin, and NMII should be provided as this will reveal the relative apicobasal position of Lmo7 in comparison with the junctional proteins.
Localization of Lmo7 mutant proteins should be described. This would help to discern whether loss of junctional localization by DUF4757, as implied by the data in Fig. 4, underlies its loss of function in inducing apical constriction.

The expression pattern of lmo7 gene is not shown or discussed. If lmo7 is involved in apical constriction, it is expected that the gene may be expressed in tissues undergoing apical constriction, such as bottle cells, neural hinge cells, or sensory placodes.
The staining of NMHIIA in control cells seems to be variable, with some showing predominantly cytoplasmic puncta (Fig. 6F') while the others displaying both junctional and cytoplasmic punctate signals (Fig. 7B'). The variability complicates the interpretation of the MO data.
It is proposed that Lmo7 induces apical constriction by promoting junctional actomyosin network. Does this imply that junctional actomyosin contractility drives apical constriction (in a fashion that resembles pulling the purse-string)? If so, this is different from the popular model suggesting an important role of medioapical actomyosin network in apical constriction in various contexts. This should be discussed in more depth.
The authors imply that F-actin may not be involved in apical constriction induced by Lmo7(242-709). No alternative mechanism is proposed to explain the phenotype. Are other cytoskeleton components involved in this process?
Since ectodermal cells in gastrula embryos do not undergo apical constriction the MO phenotype seems to suggest that Lmo7 modulates junctional tension to control ectodermal rigidity and architecture rather than apical constriction.
This idea is consistent with the data in Fig. 8 showing Lmo7-depedent dissolution of Wtip aggregates. This should be discussed in more detail. If the endogenous activity of Lmo7 is not involved in apical constriction, the title of the manuscript should be changed.

Reviewer 3

Advance summary and potential significance to field

In this manuscript, Matsuda and colleagues investigate the mechanisms of apical constriction in the Xenopus ectoderm. Specifically, they study the role of the LIM domain only protein 7 (Lmo7), which was initially identified as a binding partner of both Afadin and alpha-actinin, and whose Drosophila ortholog regulates the size of the apical domain. Lmo7 localized adjacent to, but not at, apical tight and tricellular junctions, similar to non-muscle myosin II and F-actin. Lmo7 overexpression induced formation of thick actomyosin bundles containing alpha-actinin that flanked the junctions, followed by apical constriction. Lmo7 knock down led to expanded apical surface areas and reduced junctional myosin.
Inhibiting myosin II activity disrupted Lmo7-induced tissue invagination.
Structure function analysis suggested that the DUF4757 domain and the alpha-actinin-binding domain of Lmo7 were necessary to induce apical constriction. NMIIA and NMIIB were pulled down with Lmo7 expressed in HER293T cells, with the DUF4757 domain both necessary and sufficient for the interaction between Lmo7 and the myosins. The authors show that Wtip, an Ajuba family protein that dissociates from junctions under increased tension, dissociates from junctions when Lmo7 is overexpressed, suggesting that Lmo7 causes an increase in junctional tension that could drive apical constriction.

I really enjoyed reading this manuscript, it is well-written, clear, and to the point. Some conclusions need strengthening with additional quantification, and the use of statistics could be reviewed. I also have some questions about data interpretation, and the relevance of Lmo7 during embryonic development, as most of the study is based on overexpression experiments.

Comments for the author

MAJOR
1. Figure 1E-F: the authors argue that Lmo7 and myosin colocalize close to apical junctions. This should be quantified. While I agree that both proteins share the proximity to apical junctions, from Figure 1F" it looks like myosin puncta exclude Lmo7 and vice versa? Colocalization analysis restricted to the junction would help to determine if myosin and Lmo7 colocalize or display complementary distributions at the junction. A similar comment applies to the colocalization between Lmo7 and phalloidin (Figure 3A-C).
2. Figure 8: the authors should quantify RFP-HA-Wtip junctional fluorescence in Lmo7 overexpressing and non-overexpressing cells. The junctions highlighted in panels B-D display clear

differences, but there are many other non-highlighted junctions in which the diffrence is not so obvious.
3. Figures 3 and S2: overexpression of Lmo7 induces formation of thick actomyosin bundles flanking the junctions. However, from these figures it is also apparent the presence of an medioapical actomyosin pool when Lmo7 is overexpressed. How do the authors know that the apical constriction induced by Lmo7 overexpression is a consequence of the junctional actomyosin bundles, and not the medioapical actomyosin pool (e.g. see Adam Martin's work in Drosophila gastrulation)? Do actin and myosin form medial networks apical to the junctions? Also, in Figure 8, Wtip forms medioapical puncta in cells that do not overexpress Lmo7, but seems to completely disappear from the medioapical surface in cells that overexpress Lmo7 suggesting that medioapical tension may also increase upon Lmo7 overexpression.
4. How does loss of Lmo7 function affect embryonic development? I am quite convinced based on the presented data that Lmo7 overexpression can induce apical constriction, but is that important for any developmental processes? The authors discuss that in mice, Limch1 can compensate for the loss of Lmo7. Is that the case in Xenopus too? And if so, does Lmo7 overexpression rescue double knock downs on Lmo7 and Limch1?
5. Statistics: Student's t-test is used in several places. The authors should either verify the assumption that the compared samples display normal distributions, or use non parametric tests (e.g. Mann-Whitney). Also, comparison of three or more groups should be done with the appropriate tests (e.g. Dunn's test).

MINOR
1. Figure 1E-F: the authors should add a zoom at a tricellular junctions: from panel E it seems like Lmo7 and myosin may have different localization patterns there?
2. Figures 2D, F, 4L, S1C: area units should be converted to square microns to allow readers to get a sense for the size of the cells and the changes in apical surface area.
3. Figure 3M-P: the result that Lmo7 requires Rho-ROCK-NMII signalling to induce apical constriction would be stronger if the authors quantified apical cell areas in animals overexpressing Lmo7 and one of the RNAs that they used to inhibit myosin activity.
4. Could Lmo7 recruit myosin to junctions indirectly, by recruiting Rho or ROCK?
This possibility should at least be discussed.

First revision

Author response to reviewers' comments

Matsuda et al. Responses to the reviewers’ comments

Reviewer 1 Advance summary and potential significance to field
The current study describes the LMO7 function in the apical constriction of ectodermal cells in xenopus embryos. Here, LMO7 is shown to bind Non-Muscle Myosin II A (NMIIA) through DUF4757 domain, and that LMO7 expression is required to localise NMIIA to the apical junctions. The role of LMO7 in apical size is further supported by loss-of-function experiments that contribute to the enlargement of the apical end-feet. As a read out of tissue tensions, the current study characterises the Wtip punctate and linear patterns at apical junctions following LMO7 gain-of-function. The role of LMO7 on apical constriction has been previously shown in Drosophila (Beati et al, 2018) but the current work potentially highlights the evolutionary conserved role of LMO7 between distinct animal species.

Does LMO7 loss of function and apical constriction defects impairs neural tube/plate formation or morphology? answering this question would increase the novelty of the current findings.

--- In response to this comment, we included the result of our loss-of-function study showing delayed neural tube closure in lmo7 morphants (Fig. 9H-L, Fig. S7C). This result is consistent with Lmo7 playing a role in the formation of apically constricted cells in the folding neural plate. Additionally, we note in discussion that Lmo7 may have a general function in apical domain regulation, rather than exclusively in apical constriction (the first paragraph in page 12).

Reviewer 1 Comments for the author
Points to consider:

1. Explain the changes in pigmentation in xenopus embryos.

--- We explained that a thick layer of apical F-actin in apically constricting cells is accompanied with pigment granule accumulation at the apical surface (Kurth and Hausen, 2000). For that reason, increased ectoderm pigmentation is often used as a marker of apical constriction in Xenopus embryos, e.g. (Haigo et al., 2003; Popov et al., 2018)(Page 6).

2. The field commonly uses the apical end-feet size to infer apical constriction, but other methods such as laser ablation and FRET should be complementary used. The small size of apical end-feet does not necessarily indicate apical constriction. For example, nuclei position during interkinetic nuclear migration also affects the size of the apical end-feet.

--- The revision includes additional data supporting our conclusion about apical constriction in Lmo7-expressing cells, however we did not include laser ablation or FRET due to technical reasons. Our imaging core facility does not have a pulsed laser required for efficient ablation. Access to other institutions is currently limited due to the Covid-19 pandemic. We tried 409 nm image acquisition laser, however it was not successful. Since neither laser ablation nor FRET tension sensors directly demonstrate the change in cell shape, we decided not to pursue this further. Instead, we included ectoderm cross-sections with Lmo7-expressing cells that contain expanded basolateral domain and reduced apical domain (Fig. 2E-G). These cells are not present in control ectoderm.

3. Wtip dotted / linear pattern can be a good read out for tension, but this should be demonstrated in the current study by other methods such as laser ablation. For example, when LMO7 is overexpressed and a laser ablation is performed, would this transform Wtip linear pattern into a punctate pattern?

--- Wtip belongs to the Ajuba family proteins, which relocalize to cell-cell junctions in response to tension (Ibar et al., 2018; Razzell et al., 2018). We previously observed that Wtip puncta are redistributed in the cells expressing Shroom3, a known inducer of apical constriction (Chu et al., 2018), possibly reflecting mechanical stress. We appreciate the reviewer's suggestion that we should follow up on this observation, however, the detailed investigation of Wtip properties is outside the scope of the present study. In the revised manuscript, we acknowledged the existing limitation and tuned down our conclusions in the revised text (pages 7-8).

4. the quantification and statistical section only mentions the analysis of fluorescence intensity. It lacks information regarding the type of test used in other experiments such as apical end-feet size, or whether it is paired or an
unpaired t-test, etc.

--- We included the detailed methodology for the quantification and statistical analyses in the Methods (pages 18-19).

Reviewer 2 Comments for the author
The Lmo7 protein localization is shown only in the enface view. A side view of Lmo7 localization with ZO1 (or cadherin), F-actin, and NMII should be provided, as this will reveal the relative apicobasal position of Lmo7 in comparison with the junctional proteins.

--- As requested by the reviewer, we included lateral views of ectoderm cells stained for Lmo7 as compared to other proteins: ZO-1 (Fig. 1F-G"), ß-catenin (Fig. 1H, H'), F-actin (Fig. 1P-Q") and NMIIA (Fig. 1N-O"). Lmo7 is present at the most apical area of the lateral membrane and colocalizes with ZO1, confirming its enrichment at apical junctions. We also observed Lmo7 localization at the apical cortex, which is included in the revision (Fig. 4).

Localization of Lmo7 mutant proteins should be described. This would help to discern whether loss of junctional localization by DUF4757, as implied by the data in Fig. 4, underlies its loss of function in inducing apical constriction.
--- The subcellular distribution of Lmo7 mutant proteins was included as requested (Fig. S5C-F"). Although Lmo7(242-400) had reduced localization to apical junctions, the loss of its activity is likely due to its inability to enrich NMII (Fig. S5G-H). We note that Lmo7(4A) with mutations in DUF4757 still localized to intercellular junctions (Fig. 8A, B) but no longer bound (Fig. S4A) or enriched NMII (Fig. 8A', B'), and was not able to trigger apical constriction (Fig. S4E). This question is discussed in the revised manuscript on page 9.

The expression pattern of lmo7 gene is not shown or discussed. If lmo7 is involved in apical constriction, it is expected that the gene may be expressed in tissues undergoing apical constriction, such as bottle cells, neural hinge cells, or sensory placodes.

--- The data on lmo7 expression was included in the revision (Fig. S6). RT-PCR analysis demonstrated ubiquitous expression of lmo7 at different stages of embryonic development (Fig. S6A). In situ hybridization (Fig. S6B-O) revealed lmo7 transcripts in the blastopore bottle cells at gastrulation and the neural plate. In the tailbud, lmo7 is abundant in the pharyngeal arches, the somites and the heart. Given that RNA and protein levels might not match due to post-translational regulation, future studies would need to focus on protein analysis, which we currently consider out of scope.

The staining of NMHIIA in control cells seems to be variable, with some showing predominantly cytoplasmic puncta (Fig. 6F') while the others displaying both junctional and cytoplasmic punctate signals (Fig. 7B'). The variability
complicates the interpretation of the MO data.

--- The reviewer correctly noted the variability of our NMIIA immunostaining. To address this criticism, we carried out new experiments using GFP-NMIIA, replaced the panels in question (Fig. 9A-B') and included the quantification (Fig. 9C).

It is proposed that Lmo7 induces apical constriction by promoting junctional actomyosin network. Does this imply that junctional actomyosin contractility drives apical constriction (in a fashion that resembles pulling the purse-string)? If so, this is different from the popular model suggesting an important role of medioapical actomyosin network in apical constriction in various contexts. This should be discussed in more depth.

--- The organized actomyosin bundles in Lmo7-expressing cells resemble the circumferential belt with sarcomere-like organization described for several epithelial tissues in vivo (Ebrahim et al., 2013). This observation led us to propose that Lmo7 operates via a 'purse-string' mechanism. However, this does not exclude a role for the medioapical actomyosin network as reported in various contexts, especially in Drosophila (Azevedo et al., 2011; Blanchard et al., 2010; David et al., 2010; Martin et al., 2009; Mason et al., 2013). Consistent with the latter mechanism, we found increased junctional and medioapical F-actin and NMIIA in Lmo7-expressing cells (Fig. 3 and Fig. 4). These observations have been included in the revised manuscript (pages 3-4, 6-7, 11-12).

The authors imply that F-actin may not be involved in apical constriction induced by Lmo7(242-709). No alternative mechanism is proposed to explain the phenotype. Are other cytoskeleton components involved in this process?

--- Lmo7(242-709) caused apical constriction but did not increase F-actin association with AJs at least in our current assay. We did not intend to imply that F-actin has no role in apical constriction and amended the text to clarify our view (page 9 and page 13).

Since ectodermal cells in gastrula embryos do not undergo apical constriction, the MO phenotype seems to suggest that Lmo7 modulates junctional tension to control ectodermal rigidity and architecture rather than apical constriction.
This idea is consistent with the data in Fig. 8 showing Lmo7-depedent dissolution of Wtip aggregates. This should be discussed in more detail. If the endogenous activity of Lmo7 is not involved in apical constriction, the title of the manuscript should be changed.

--- We fully agree with the referee that our results support the view that Lmo7 acts on cell tension and rigidity rather than specifically on apical constriction. Nevertheless, our new observation with lmo7 morphants exhibiting delayed neural tube closure is also consistent with deficient apical constriction (Fig. 9H-L, S7C). We discuss both possibilities in the revised manuscript (page 12). We also changed the title to “Lmo7 recruits myosin II heavy chain to regulate actomyosin contractility and apical domain size in Xenopus ectoderm”.

Reviewer 3 Advance summary and potential significance to field
In this manuscript, Matsuda and colleagues investigate the mechanisms of apical constriction in the Xenopus ectoderm. Specifically, they study the role of the LIM domain only protein 7 (Lmo7), which was initially identified as a binding partner of both Afadin and alpha-actinin, and whose Drosophila ortholog regulates the size of the apical domain. Lmo7 localized adjacent to, but not at, apical tight and tricellular junctions, similar to non-muscle myosin II and F-actin. Lmo7 overexpression induced formation of thick actomyosin bundles containing alpha-actinin that flanked the junctions, followed by apical constriction. Lmo7 knock down led to expanded apical surface areas and reduced junctional myosin. Inhibiting myosin II activity disrupted Lmo7-induced tissue invagination. Structure function analysis suggested that the DUF4757 domain and the alpha- actinin-binding domain of Lmo7 were necessary to induce apical constriction. NMIIA and NMIIB were pulled down with Lmo7 expressed in HER293T cells, with the DUF4757 domain both necessary and sufficient for the interaction between Lmo7 and the myosins. The authors show that Wtip, an Ajuba family protein that dissociates from junctions under increased tension, dissociates from junctions when Lmo7 is overexpressed, suggesting that Lmo7 causes an increase in junctional tension that could drive apical constriction.

I really enjoyed reading this manuscript, it is well-written, clear, and to the point. Some conclusions need strengthening with additional quantification, and the use of statistics could be reviewed. I also have some questions about data interpretation, and the relevance of Lmo7 during embryonic development, as most of the study is based on overexpression experiments.

Reviewer 3 Comments for the author
MAJOR
1. Figure 1E-F: the authors argue that Lmo7 and myosin colocalize close to apical junctions. This should be quantified. While I agree that both proteins share the proximity to apical junctions, from Figure 1F" it looks like myosin puncta exclude Lmo7 and vice versa? Colocalization analysis restricted to the junction would help to determine if myosin and Lmo7 colocalize or display complementary distributions at the junction. A similar comment applies to the colocalization between Lmo7 and phalloidin (Figure 3A-C).

--- We quantified the distribution of Lmo7, NMIIA and F-actin relative to the position of the plasma membrane (Fig. 1R) and included a relevant schematic (Fig. 1S).

2. Figure 8: the authors should quantify RFP-HA-Wtip junctional fluorescence in Lmo7 overexpressing and non-overexpressing cells. The junctions highlighted in panels B-D display clear differences, but there are many other non-highlighted junctions in which the difference is not so obvious.

--- The relevant panels have been changed (Fig. 5I-K”) and the requested quantification of RFP-HA-Wtip fluorescence was added (Fig. 5M).

3. Figures 3 and S2: overexpression of Lmo7 induces formation of thick actomyosin bundles flanking the junctions. However, from these figures it is also apparent the presence of an medioapical actomyosin pool when Lmo7 is overexpressed. How do the authors know that the apical constriction induced by Lmo7 overexpression is a consequence of the junctional actomyosin bundles, and not the medioapical actomyosin pool (e.g. see Adam Martin's work in Drosophila gastrulation)? Do actin and myosin form medial networks apical to the junctions? Also, in Figure 8, Wtip forms medioapical puncta in cells that do not overexpress Lmo7, but seems to completely disappear from the medioapical surface in cells that overexpress Lmo7, suggesting that medioapical tension may also increase upon Lmo7 overexpression.

--- Thank you for this important comment. We included the data showing that Lmo7 localizes to both apical junctions and the apical cortex and promotes the formation of the medioapical actomyosin network (Fig. 4). We acknowledge that the present results do not allow us to discriminate whether apical constriction is caused by the circumferential or medioapical actomyosin contractions (pages 3-4, 6-7, 11-12).

4. How does loss of Lmo7 function affect embryonic development? I am quite convinced based on the presented data that Lmo7 overexpression can induce apical constriction, but is that important for any developmental processes? The authors discuss that in mice, Limch1 can compensate for the loss of Lmo7. Is that the case in Xenopus too? And if so, does Lmo7 overexpression rescue double knock downs on Lmo7 and Limch1?

--- As we noted in our responses to other referees, Lmo7 morphants exhibited moderate neural plate closure defects (Figs. 9H-L, S7C), consistent with its proposed role in apical constriction. Nevertheless, we acknowledged that our data are also consistent with a more general function of Lmo7 in apical domain size control (Page 12).

--- We also expanded the discussion of Limch1, a homologue of Lmo7. Whether Limch1 plays a redundant role in actomyosin contractility in mice or Xenopus is currently unknown. Whereas the detailed characterization of Limch1 is warranted, we feel that it is beyond the scope of the present study.

5. Statistics: Student's t-test is used in several places. The authors should either verify the assumption that the compared samples display normal distributions, or use non parametric tests (e.g. Mann-Whitney). Also, comparison
of three or more groups should be done with the appropriate tests (e.g. Dunn's test).

--- We have re-done the statistical analyses as recommended: the Mann-Whitney U-test for two groups and the Dunn’s test using a Bonferonni correction for the p-values.

MINOR
1. Figure 1E-F: the authors should add a zoom at a tricellular junctions: from panel E it seems like Lmo7 and myosin may have different localization patterns there?

--- A magnified fluorescence image of a tricellular junction was added (Fig. 1K-K”).

2. Figures 2D, F, 4L, S1C: area units should be converted to square microns to allow readers to get a sense for the size of the cells and the changes in apical surface area.

--- The units were converted to μm2 as suggested (Figs. 1D, 1I, 5G, 6M, S1C).

3. Figure 3M-P: the result that Lmo7 requires Rho-ROCK-NMII signalling to induce apical constriction would be stronger if the authors quantified apical cell areas in animals overexpressing Lmo7 and one of the RNAs that they used to inhibit myosin activity.

--- Quantification of apical domain size in cells expressing Lmo7 and CA-Mypt was added as suggested (Fig. 5E-G).

4. Could Lmo7 recruit myosin to junctions indirectly, by recruiting Rho or ROCK? This possibility should at least be discussed.

--- This discussion was expanded to include this possibility (page 12, last paragraph).

References

Azevedo, D., Antunes, M., Prag, S., Ma, X., Hacker, U., Brodland, G. W., Hutson, M. S., Solon, J. and Jacinto, A. (2011). DRhoGEF2 regulates cellular tension and cell pulsations in the Amnioserosa during Drosophila dorsal closure. PLoS One 6, e23964.

Blanchard, G. B., Murugesu, S., Adams, R. J., Martinez-Arias, A. and Gorfinkiel, N. (2010). Cytoskeletal dynamics and supracellular organisation of cell shape fluctuations during dorsal closure. Development 137, 2743-52.
Chu, C. W., Xiang, B., Ossipova, O., Ioannou, A. and Sokol, S. Y. (2018). The Ajuba family protein Wtip regulates actomyosin contractility during vertebrate neural tube closure. J Cell Sci 131.
David, D. J., Tishkina, A. and Harris, T. J. (2010). The PAR complex regulates pulsed actomyosin contractions during amnioserosa apical constriction in Drosophila. Development 137, 1645-55.
Ebrahim, S., Fujita, T., Millis, B. A., Kozin, E., Ma, X., Kawamoto, S., Baird, M. A., Davidson, M., Yonemura, S., Hisa, Y. et al. (2013). NMII forms a contractile transcellular sarcomeric network to regulate apical cell junctions and tissue geometry. Curr Biol 23, 731-6.
Haigo, S. L., Hildebrand, J. D., Harland, R. M. and Wallingford, J. B. (2003). Shroom induces apical constriction and is required for hingepoint formation during neural tube closure. Curr Biol 13, 2125-37.
Ibar, C., Kirichenko, E., Keepers, B., Enners, E., Fleisch, K. and Irvine, K. D. (2018). Tension-dependent regulation of mammalian Hippo signaling through LIMD1. J Cell Sci 131.
Kurth, T. and Hausen, P. (2000). Bottle cell formation in relation to mesodermal patterning in the Xenopus embryo. Mech Dev 97, 117-31.
Martin, A. C., Kaschube, M. and Wieschaus, E. F. (2009). Pulsed contractions of an actin-myosin network drive apical constriction. Nature 457, 495-9.
Mason, F. M., Tworoger, M. and Martin, A. C. (2013). Apical domain polarization localizes actin-myosin activity to drive ratchet-like apical constriction. Nat Cell Biol 15, 926-36.
Popov, I. K., Ray, H. J., Skoglund, P., Keller, R. and Chang, C. (2018). The RhoGEF protein Plekhg5 regulates apical constriction of bottle cells during gastrulation. Development 145.
Razzell, W., Bustillo, M. E. and Zallen, J. A. (2018). The force-sensitive protein Ajuba regulates cell adhesion during epithelial morphogenesis. J Cell Biol 217, 3715-3730.

Second decision letter

MS ID#: DEVELOP/2021/200236

MS TITLE: Lmo7 recruits myosin II heavy chain to regulate actomyosin contractility and apical domain size in Xenopus ectoderm

AUTHORS: Miho Matsuda, Sergei Y Sokol, and Chih-Wen Chu

You will be pleased to hear that the referees are happy with your revisions and there is just one issue for you to consider 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 1

Advance summary and potential significance to field

For the first time this study investigates the lmo7 function in apical constriction in xenopus ectoderm. The experimental findings highlight for the first time the role of lmo7 in neural tube morphogenesis. I found that the revised version of the manuscript is greatly improved. The observations, figures, etc and data analysis are robust.

Comments for the author

The majority of my concerns have been addressed with experiments or in authors reply.

Reviewer 2

Advance summary and potential significance to field

The manuscript by Matsuda et al. describes functional and mechanistical studies of Lmo7 in regulating actomyosin accumulation at the cell junction and apical cortex in Xenopus embryonic ectodermal cells. The authors show that Lmo7 is localized near the junctions of adjacent cells and can induce ectodermal cell apical constriction when ectopically expressed at high doses. Domain analysis reveals that DUF4757 and alpha-actinin binding motif are both required for induction of apical constriction. Lmo7 binds and recruits NMIIA to cell junctions to promote junctional actomyosin bundles. Knockdown of Lmo7 causes delay in neural tube closure. The authors conclude that Lmo7 recruits NMII and regulates its incorporation into actomyosin networks to control apical domain size.

Comments for the author

The authors have addressed all my concerns satisfactorily in this revised manuscript by adding both additional data and more in depth and balanced discussion. I therefore recommend publication of the manuscript.

Reviewer 3

Advance summary and potential significance to field

The authors have nicely addressed most of my concerns. I was a bit disappointed with the quantification of colocalization in figure 1R: is this from a single junction? If so, the authors should conduct the measurement in many more. If it is an average, the error in the measurement should be displayed. Based on the data provided, Lmo7 seems to colocalize more strongly with F-actin than with myosin and I think that that should be quantified across multiple junctions. The authors may want to consider using Pearson's correlation at the junction itself to compare colocalization levels.

Comments for the author

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Second revision

Author response to reviewers' comments

Matsuda et al. Responses to the reviewers' comments

Reviewer 3 comments:
The authors have nicely addressed most of my concerns. I was a bit disappointed with the quantification of colocalization in figure 1R: is this from a single junction? If so, the authors should conduct the measurement in many more. If it is an average, the error in the measurement should be displayed. Based on the data provided, Lmo7 seems to colocalize more strongly with F-actin than with myosin, and I think that that should be quantified across multiple junctions. The authors may want to consider using Pearson's correlation at the junction itself to compare colocalization levels.

Responses:
The original quantification in Figure 1R was from a single junction. In the revised manuscript, the measurement from many more junctions was included. We also included error bars and Spearman's

correlation coefficient because our data sets fit better than to Pearson's linear relationship. As suggested by the reviewer, Lmo7 co-localized better with F-actin than myosin II. We have modified the figure legend accordingly (Page 26).

Third decision letter

MS ID#: DEVELOP/2021/200236

MS TITLE: Lmo7 recruits myosin II heavy chain to regulate actomyosin contractility and apical domain size in Xenopus ectoderm

AUTHORS: Miho Matsuda, Sergei Y Sokol, and Chih-Wen Chu
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.