Ciona embryonic tail bending is driven by asymmetrical notochord contractility and coordinated by epithelial proliferation

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AUTHORS: Qiongxuan LU, Yuan Gao, Yuanyuan Fu, Hongzhe Peng, Wenjie Shi, Bo Li, Xi-Qiao Feng, and Bo Dong

I sincerely apologise for the long delay before coming back to you. 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, which in my view could be addressed and recommend a revision of your manuscript before we can consider publication. If you are able to revise the manuscript along the lines suggested, which may involve further experiments, 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' major concerns. Please also note that Development will normally permit only one round of major revision.

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 authors examine how ascidian embryos get a bent tail. They find that within the posterior part of the embryo the notochord is stiffer than muscles and the outer epithelium, and that the actomyosin cytoskeleton accumulates more prominently in the ventral part of the notochord compared to its dorsal part. Furthermore, their data show that notochord-specific expression of a non-phosphorylatable version of myosin-2 regulatory light chain, or of cofilin induces tail bending defects. Finally, they observe that the dorsal part of the epidermis proliferates at a higher rate compared to the ventral part, but that this differential proliferation rate does not plays a major role in bending; indeed the aspect ration of these cells is not very different and does not change much over time. They use this data to feed a finite element model suggesting that tail bending results mostly from the asymmetric contractility of the ventral notochord. This work makes a strong candidate for publication in Development because it dissects a fundamental process from the mechanical standpoint and has potential implications for the development of vertebrate embryos.

Comments for the author

The experiments are well conducted and quantified, although I have a few rather minor queries for the authors. My two main reservations at this point concern the English grammar, which they should improve all along the manuscript, and the model.

Model:
I could not really see how in the model they take into account the fact that tension is asymmetric and more pronounced ventrally, as it is not apparent in the equations (2) and (3), or else not properly explained. Additionally, the authors take the value of the constriction force in the Drosophila amnioserosa, which could be acceptable with some validation either experimental or by varying that parameter in some systematic manner between extreme values observed in different in vivo settings.

Minor points:
1- The enrichment in phosphorylated rMLC ventrally is not huge. What is the statistical significance of this enrichment?
2- The authors conclude (line 217) “our results reveal that the asymmetrical actomyosin contractility is required for notochord and the tail bending properly”. I agree that this conclusion is likely, but will encourage the authors to be slightly cautious. What the authors have found is that phospho-rMLC is very slightly enriched ventrally (see previous point) and that expression of a non-phosphorylatable rMLC variant affects bending, but they do not know which myosin II structure is affected. In particular, an earlier paper by some of the same authors had reported the prominent role of an equatorial actomyosin ring in elongating the notochord.
3- Line 268: it is Euler-Bernouilli.
4- Line 270: please define Iz.
5- Line 331: please be more precise and say “by expressing myosin II and Cofilin mutated variants.”
6- Line 471: space missing before the parenthesis.
7- Figure 2C-D: what is sample size?
8- TableS1/FigS3C versus Fig4D: the length of the dorsal epidermis appears to be roughly 2x longer than the ventral epidermis from stage 19 onwards (FigS3C), yet the difference in cell number or proliferation rate does not seem as important. Did the author check whether the two values match?

Reviewer 2

Advance summary and potential significance to field

In this paper, Lu and colleagues investigate the mechanisms that govern tail bending during ascidian development. Using simple micromanipulation experiments, the authors establish that tail bending is independent of confinement by the chorion, that the force-generating mechanism

resides within the anterior region of the tail itself. They show that filamentous actin (F-actin) and activated Myosin II are enriched on the ventral boundary of the notochord and adjacent endodermal strand. Notochord-specific perturbations to myosin activity and a factor (Cofilin) that controls F-actin disassembly, both inhibit tail bending. They show further that tail bending is associated with higher rates of cell division within the dorsal vs ventral epidermis, and blocking cell divisions globally, or just within the dorsal epidermis, inhibit tail bending. They consider the possibilities either (1) that epidermal cell divisions either generate active stress to help drive cell bending or (2) relieve stress due to forces produced in neighboring tissues. Using AFM measurements of relative tissue stiffness and finite element modeling, the authors argue against possibility (1), and conclude that active forces produced by differential deployment of F-actin and Myosin II within the notochord drive tail bending.

These results provide an interesting window into how a morphological feature of a whole embryo can be controlled through mechanical interplay between between force generation and dissipation across multiple tissues. As such, they should be of general interest to the developmental biology community. However, there are some issues that need to be addressed before I would support publication.

Comments for the author

(1) The notochord must change shape to accommodate bending of the tail, and this will necessarily produce a difference in dorsal vs ventral lengths as documented in Figure 2B. Thus the existence of a difference between DL vs VL does not necessarily imply anything about an active role for notochord in tail bending. For this observation to have any force, the observed difference would have to be compared to the difference predicted for a scenario in which the notochord were passively deformed by imposed bending moment. There is also a taper of the notochord, described by Veeman's group, that would complicate this analysis

(2) The authors assume that the ventral enrichments of F-actin and Myosin II are in the notochord and not the adjacent endodermal strand cells? This seems likely, but it should be confirmed. Are markers for Myosin II or F-actin asymmetrically localized when expressed in the notochord?

(3) Related to (2), this may be beyond the scope of this paper, but it would be cool if the authors could ablate the endodermal strand precursor cells and observe the effect on ventral accumulation of F-actin and Myosin II in the notochord and the overall effect on tail bending.

(4) The cofilin experiment is unsatisfactory for several reasons. First, the authors do not describe clearly what effect the S5A mutation is predicted to have on F-actin, nor do they document what effect this perturbation has on F-actin or Myosin II in the notochord. Without this information, it is impossible to interpret this experiment in a meaningful way.

(5) The authors describe dorsal vs ventral differences in cell proliferation rates, But the effects of these divisions would be very different, depending on the orientations of the divisions – i.e. if the orientations are random, and overall cell volume is conserved, cell divisions may simply partition the same volume into smaller packets, with little effect on tissue level dynamics, whereas oriented divisions could drive local tissue extension, and/or relieve local tissue resistance. Have the orientations of these epidermal divisions been characterized in previous work? If so, the authors should cite this work and factor it into their interpretation.
If not, it would be important to characterize the orientations of these divisions themselves.

(6) If the cell division asymmetry is a consequence of forces produced by the notochord then that asymmetry should disappear when notochord contractility is inhibited.

(7) I did not find the mechanical analysis presented in figure 5 very compelling. First , the measured difference in stiffness notochord vs epidermis does not in any way rule out the possibility that epidermis could provide part of the asymmetric driving force for tail bending.

Second, without a much better description of the finite element simulations, it is impossible for a reader to draw any meaningful conclusions from the results. What exact assumptions underlie the

notochord driving vs epidermis driving scenarios? In what sense (if at all) are these mutually exclusive possibilities? In particular, it has already been shown that, as the tail bends, it is also elongating, and this elongation is powered by active notochord extension. So we already know that the notochord is generating an active force to drive the extension of the tail, and that this active force will tend to stretch the entire epidermis. In principle, tail bending could be explained by superimposing upon this active extension force either a difference in actomyosin contractility along the notochord boundary, or a dorsal vs ventral asymmetry in active spreading of the epidermis. Importantly, both scenarios could lead to the observed decreased in height of epidermal cells, depending on the rates and patterns of cell divisions. Therefore, I do not see how observed changes in epidermal cell shapes can be used to distinguish these two scenarios.

(8) More generally, if the finite element simulations do not explicitly capture the combination of tail extension and bending, I do not see how their predictions can be compared in a meaningful way to the experimental observations/measurements. To be clear, I support the use of modeling to help interpret these data. I just think the authors need to be more clear about the modeling assumptions, if they have not done so, they need to consider simultaneously the dynamics of tail extension and bending. In fact, I think that doing this more explicitly would provide a richer context in which to interpret e.g. the perturbation experiments shown in Figure 4.

(9) I think there are some simple ways in which the authors could further assess the relative magnitudes and autonomy of contributions of notochord and epidermis to tail bending that do not rely on indirect inference from mechanical models. For example, if the authors prevent notochord extension (e.g. by inhibiting prickle or disheveled), do the asymmetries in epidermal cell divisions persist? In the aphidicolin (or cdc-45) experiments, what happens to the asymmetries in F-actin and Myosin II in the notochord? Do they persist? When the tail bends in the opposite direction, is this associated with a change in F-actin and Myosin II distributions? If not, then doesn't this imply that (at least in this perturbed condition) that actomyosin asymmetries are not determining the direction of tail bending?

(10) The paper is full of awkward phrasing, misspelled and misused words. All of this could be easily solved by asking a native English-speaking colleague for editorial input.

First revision

Author response to reviewers' comments

Point-by-point response

We appreciate the time and effort that the referees took to thoroughly review our manuscript and thank them for their constructive criticisms and insightful suggestions. We believe that we have addressed all their concerns by performing additional experiments and rephrasing the respective aspect of the manuscript.

Reviewer 1 Advance Summary and Potential Significance to Field:
The authors examine how ascidian embryos get a bent tail. They find that within the posterior part of the embryo the notochord is stiffer than muscles and the outer epithelium, and that the actomyosin cytoskeleton accumulates more prominently in the ventral part of the notochord compared to its dorsal part. Furthermore, their data show that notochord-specific expression of a non-phosphorylatable version of myosin-2 regulatory light chain, or of cofilin induces tail bending defects. Finally, they observe that the dorsal part of the epidermis proliferates at a higher rate compared to the ventral part, but that this differential proliferation rate does not plays a major role in bending; indeed, the aspect ratio of these cells is not very different and does not change much over time. They use this data to feed a finite element model suggesting that tail bending results mostly from the asymmetric contractility of the ventral notochord. This work makes a strong candidate for publication in Development because it dissects a fundamental process from the mechanical standpoint and has potential implications for the development of vertebrate embryos.

Reviewer 1 Comments for the Author:
The experiments are well conducted and quantified, although I have a few rather minor queries for the authors. My two main reservations at this point concern the English grammar, which they should improve all along the manuscript, and the model.
Response: Thanks for the suggestions. The English writing of manuscript has been checked and edited carefully by a native English speaker.

Model:
I could not really see how in the model they take into account the fact that tension is asymmetric and more pronounced ventrally, as it is not apparent in the equations (2) and (3), or else not properly explained. Additionally, the authors take the value of the constriction force in the Drosophila amnioserosa, which could be acceptable with some validation either experimental or by varying that parameter in some systematic manner between extreme values observed in different in vivo settings.

Response: During the tail bending, both F-actin and Myosin-II enrich in the ventral notochord, which induces asymmetric tension. The moment arising from the tension further bends the tail. To evaluate whether the asymmetric tension of notochord is sufficient to drive the tail bending or not, we treat the tail as a multi-layered beam. The asymmetric tension is treated as a contraction stress σ (or equivalently contraction strain) at the ventral side. The bending moment and the corresponding rotational angle of the beam can be derived from Eqs. (2) and (3). Because the exact value of σ is hard to measure from experiments, we take the value of *Drosophila* for reference. Though this reference value may deviate from the real value of *Ciona*, the underlying mechanical mechanisms are similar.

Minor points:
1-The enrichment in phosphorylated rMLC ventrally is not huge. What is the statistical significance of this enrichment?

Response: We agree with the reviewer that the enrichment of the phosphorylated MLC at the ventral side of the notochord is not prominent compared to F-actin (Figure 2D, F). However, as suggested, we compared the fluorescent intensity of the phosphorylated MLC at the dorsal and ventral side of notochord by t-test. The results showed that the difference was statistical significance at the stage 17, stage 19, and stage 21, respectively, but not for the stage 22 (See the following Table 1).

Table 1. Fluorescent intensity comparison of the phosphorylated MLC between the dorsal and ventral sides of notochord at different stages

Stages	Stage 17	Stage 19	Stage 21	Stage 22
P value	0.008301	9.20475E-10	3.29E-05	0.775656

2-The authors conclude (line 217) “our results reveal that the asymmetrical actomyosin contractility is required for notochord and the tail bending properly”. I agree that this conclusion is likely, but will encourage the authors to be slightly cautious. What the authors have found is that phospho-rMLC is very slightly enriched ventrally (see previous point) and that expression of a non-phosphorylatable rMLC variant affects bending, but they do not know which myosin II structure is affected. In particular, an earlier paper by some of the same authors had reported the prominent role of an equatorial actomyosin ring in elongating the notochord.

Response: Thank the reviewer for bringing up this important point. The ventral enrichment of actomyosin and the formation of equatorial actomyosin ring in notochord are temporal-spatial difference. Our data clearly showed that the ventral enrichment of actomyosin was more prominent at the early stage (stage 17) and became symmetry gradually at a later stage (stage 22) (Figure 2C-F), whereas the accumulation of equatorial actomyosin is more prominent from stage 22 onward (Sehring et al., 2015). The analysis of tail-bent defects by the expression of MLC or cofilin mutants were performed at early stages (stage 17-21, Figure 3). Therefore, it’s reasonable to conclude that

the mutants mainly affected the ventrally polarized actomyosin and thus regulated the tail-bending processes.

Of course, as the reviewer pointed that, we could not rule out of the possibility that the expression of MLC mutants may also affect the formation of the equatorial actomyosin ring and other actin structures. So, we have therefore modified our statement (line 218-220 in the revised manuscript).

3-Line 268: it is Euler-Bernouilli.
Response: Thanks. The typo has been corrected as "Euler-Bernoulli" (line 287 in the revised manuscript).

4-Line 270: please define Iz.
Response: The symbol "Iz" denotes the moment of inertia in the multi-layered beam. We have added its definition in revision (line 291 in the revised manuscript).

5-Line 331: please be more precise and say "by expressing myosin II and Cofilin mutated variants."
Response: Following this suggestion, we have modified our statement (line 347-350 in the revised manuscript).

6-Line 471: space missing before the parenthesis.
Response: This has been corrected.

7-Figure 2C-D: what is sample size?
Response: The number of samples used in each panel of Fig. 2C are 13, 12, 13, and 6, respectively; in each panel of Fig. 2D are 6, 7, 6, and 9, respectively. The information has been provided in the main text (line 167 and 180) and the figure legend (line 802 and 804) in the revision.

8-TableS1/FigS3C versus Fig4D: the length of the dorsal epidermis appears to be roughly 2x longer than the ventral epidermis from stage 19 onwards (FigS3C), yet the difference in cell number or proliferation rate does not seem as important. Did the author check whether the two values matches?
Response: We agree with the reviewer. The A-P length of dorsal midline of tail epidermis is roughly two times longer than the ventral counterpart (Table S1/FigS3C) at stage 19. We interpret this difference mainly from the following two aspects:
1) Differential cell division rate. Our BrdU staining results showed that the whole dorsal epidermis cells were divided faster than that in ventral counterpart (Fig. 4D). When counting the number of dorsal and ventral midlines of tail epidermis (middle section), we found the cell number in dorsal was average 1.6x more than the ventral counterpart (Table S1).
2) Differential cell shape. By measuring the A-P length (apical domain) of individual cell in dorsal or ventral midline of tail epidermis, we found that the AP length of a single dorsal epidermis cell was average 1.3x longer than that in the ventral ones from stage 19 onwards (Table S1).
Based on above two aspects, we interpret that both the differential cell proliferation (cell number) and cell shape change (stretching along A-P axis) contribute to the longer length of the dorsal epidermis.

Reviewer 2 Advance Summary and Potential Significance to Field:
In this paper, Lu and colleagues investigate the mechanisms that govern tail bending during ascidian development. Using simple micromanipulation experiments, the authors establish that tail bending is independent of confinement by the chorion, that the force-generating mechanism resides within the anterior region of the tail itself. They show that filamentous actin (F-actin) and activated Myosin II are enriched on the ventral boundary of the notochord and adjacent endodermal strand. Notochord-specific perturbations to myosin activity and a factor (Cofilin) that controls F-actin disassembly, both inhibit tail bending. They show further that tail bending is associated with higher rates of cell division within the dorsal vs ventral epidermis, and blocking cell divisions globally, or just within the dorsal epidermis, inhibit tail bending. They consider the possibilities either (1) that epidermal cell divisions either generate active stress to help drive cell bending or (2) relieve stress due to forces produced in neighboring tissues. Using AFM measurements of relative tissue stiffness and finite element modeling, the authors argue against possibility (1), and conclude that active forces produced by differential deployment of F-actin and Myosin II within the notochord drive tail bending. These results provide an interesting window into how a morphological feature of a whole embryo can be controlled through mechanical interplay between force generation and dissipation across multiple tissues. As

such, they should be of general interest to the developmental biology community. However, there are some issues that need to be addressed before I would support publication.

Reviewer 2 Comments for the Author:
(1) The notochord must change shape to accommodate bending of the tail, and this will necessarily produce a difference in dorsal vs ventral lengths as documented in Figure 2B. Thus the existence of a difference between DL vs VL does not necessarily imply anything about an active role for notochord in tail bending. For this observation to have any force, the observed difference would have to be compared to the difference predicted for a scenario in which the notochord was passively deformed by imposed bending moment. There is also a taper of the notochord, described by Veeman's group, that would complicate this analysis.

Response: We agree with the reviewer that the length difference between DL and VL is unnecessary for supporting the active role of notochord in tail-bending. In Figure 2B, we intended to bring up the notice of the "bending cylindrical" shape of notochord in the bent tail (especially for later stages). Such wedge shape suggests the ventral constriction of notochord cells. This is consistent with the observation of ventral enrichment of actomyosin. We therefore suggest that notochordal cells play an active role in tail-bending through the asymmetrical actomyosin contractility.
The reasons that we exclude the possibility of the passive deformation of notochord caused the ventral accumulation of actomyosin are based on the following two facts:
1) At stage 17, when notochord was not or very less bent, actomyosin already showed significantly asymmetric accumulation in the ventral edge of notochord (first panel in Fig. 2C), suggesting the ventral enrichment of actomyosin happens before the bending process.
2) The difference of the actomyosin in dorsal and ventral side of notochord decreased gradually from stage 17 to 22 (Fig. 2E and 2F), when the bending angle was reversely increased, suggesting the ventral enrichment of actomyosin did not result from the bending process.
We agree the reviewer that there may exist several mechanisms underlying the "bending cylindrical" shape of notochord. Previous works by Veeman and Smith suggest that the sibling cell volume asymmetries, the timing of intercalation, and the differential rates of notochord narrowing are involved in the formation of a taper notochord (Veeman and Smith, 2013). It will be very intriguing to investigate whether or how the asymmetrical actomyosin contractility contributes to the taper of the notochord in the future.

(2) The authors assume that the ventral enrichments of F-actin and Myosin II are in the notochord and not the adjacent endodermal strand cells? This seems likely, but it should be confirmed. Are markers for Myosin II or F-actin asymmetrically localized when expressed in the notochord?
Response: As suggested, we have generated two *in vivo* markers eBra>lifeact-GFP and eBra>MLC-GFP, which are commonly used to specifically label the notochord's F-actin and Myosin II, respectively (Denker et al., 2015; Dong et al., 2011). But unfortunately, both markers were failed to reproduce the ventral polarity of actomyosin in notochord, as revealed by phalloidin and pS19-MRLC antibody staining (Figure 2C-D). We assumed that two possible technical-problems: Firstly, the promoter activity is not strong sufficient to express the fusion proteins at the early tailbud stage. Secondly, overexpression of these *in vivo* marker may produce artificial effect. Therefore, overexpression of actin or myosin markers proves to be difficult to help us to exactly distinguish the asymmetric actin-originated tissues.
Then, we repeated the phalloidin staining experiments and carefully examined all the samples. Eventually, in dozens of samples, we clearly saw that the F-actin was indeed enriched at the ventral side of the notochord (see Fig. S1B in Supplementary data).
To further confirm our observation, we searched the literature and found that the strong enrichment of F-actin in ventral side of notochord (red arrow head) was present in the previous publications (See insert Figure, cited from (Hotta et al., 2007). The red and green arrowheads were added by us, green arrow head indicates the endodermal strand cells).

We have removed unpublished data provided for the referees in confidence.

(3) Related to (2), this may be beyond the scope of this paper, but it would be cool if the authors could ablate the endodermal strand precursor cells and observe the effect on ventral accumulation of F-actin and Myosin II in the notochord and the overall effect on tail bending.

Response: We thanks the reviewer for the suggestion. As mentioned already in point (2), our data clearly showed that the F-actin was enriched at the ventral side of the notochord. Because the notochord is the stiffest tissue among the tail tissues, we focus on understanding the role of notochord during the tail-bending process and how the ventral enrichment of actomyosin contributes to the notochord and hence the tail-bending.
As the reviewer pointed out, it will be very cool if we could address the effects of other tissues on the bending processes. However, ablation of endodermal strand precursor cells is very hard. Although we could not address this question experimentally, we have added some discussions in the *Discussion* section (line 382-387 in the revised manuscript).

(4) The cofilin experiment is unsatisfactory for several reasons. First, the authors do not describe clearly what effect the S5A mutation is predicted to have on F-actin, nor do they document what effect this perturbation has on F-actin or Myosin II in the notochord. Without this information, it is impossible to interpret this experiment in a meaningful way.

Response: We provide the detailed description on the cofilin experiments. Cofilin is a highly conserved actin binding protein, playing roles in actin dynamics (Lappalainen and Drubin, 1997). In mammalian, phosphorylation of cofilin at serine 3 prevents its binding from actin and thereby stabilizes the F-actin. Overexpression of the nonphosphorylatable cofilin mutant cofilin S3A is able to block the ability of RhoA to stimulate actin polymerization (Arber et al., 1998; Sotiropoulos et al., 1999). The *Ciona* has only one cofilin gene, in which serine 5 is equivalent to the serine 3 in mammalian cofilin (Sehring et al., 2014). We therefore made *Ciona* cofilin S5A to mimic the non-phosphorylated cofilin serving as a dominant-negative mutant of cofilin. In the current study, we explained that expression of the cofilin S5A mutant affected tail-bending via the disruption of the ventral F-actin enrichment and hence the asymmetrical actomyosin contractility of notochord. The above information has been added in the revised manuscript (line 202-212 in the revised manuscript).

(5)The authors describe dorsal vs ventral differences in cell proliferation rates, But the effects of these divisions would be very different, depending on the orientations of the divisions – i.e. if the orientations are random, and overall cell volume is conserved, cell divisions may simply partition the same volume into smaller packets, with little effect on tissue level dynamics, whereas oriented divisions could drive local tissue extension, and/or relieve local tissue resistance. Have the orientations of these epidermal divisions been characterized in previous work? If so, the authors should cite this work and factor it into their interpretation. If not, it would be important to characterize the orientations of these divisions themselves.

Response: We thank the reviewer for bringing up this important point. We agree the reviewer that the analysis of the orientation of cell division is crucial for our interpretation about the role of differential cell proliferation in tail bending. Characterization of the orientations of tail epidermis division have been done by Ogura et al. (Ogura et al., 2011) and Negishi et al. (Negishi et al., 2016), previously. According to their works, the tail epidermal cells tend to divide parallel to the A-P axis during the early tailbud stages. Thanks for these pioneer and excellent works, we interpret that the orientation of cell division may drive the epidermis tissue extension and relieve the local tissue resistance along the A-P axis during the tail-bending process. As suggested, we have incorporated this information into our revised manuscript (line 369-373 in the revised manuscript) and cited the references accordingly.

(6) If the cell division asymmetry is a consequence of forces produced by the notochord, then that asymmetry should disappear when notochord contractility is inhibited.

Response: In response to this comment, we examined all the embryos in notochord contractility inhibition experiments (either mutants or chemical inhibitor treatment) in this study and our previous works. We found that no significantly additional epidermis cells in the dorsal side. These observations indicate that these two processes are coordinated and it is likely that the signaling that derives the asymmetrical cell division comes from the notochord contractility. This signaling is produced either via the interaction of the chemical molecules or through the simple mechanical transduction. We have added relevant discussion in Discussion part (line 373-376).

(7) I did not find the mechanical analysis presented in figure 5 very compelling. First, the measured difference in stiffness notochord vs epidermis does not in any way rule out the possibility that

epidermis could provide part of the asymmetric driving force for tail bending. Second, without a much better description of the finite element simulations, it is impossible for a reader to draw any meaningful conclusions from the results. What exact assumptions underlie the notochord driving vs epidermis driving scenarios? In what sense (if at all) are these mutually exclusive possibilities? In particular, it has already been shown that, as the tail bends, it is also elongating, and this elongation is powered by active notochord extension. So we already know that the notochord is generating an active force to drive the extension of the tail, and that this active force will tend to stretch the entire epidermis. In principle, tail bending could be explained by superimposing upon this active extension force either a difference in actomyosin contractility along the notochord boundary, or a dorsal vs ventral asymmetry in active spreading of the epidermis. Importantly, both scenarios could lead to the observed decreased in height of epidermal cells, depending on the rates and patterns of cell divisions. Therefore, I do not see how observed changes in epidermal cell shapes can be used to distinguish these two scenarios.

Response: During the tail bending, the exerted forces on the epidermal cells are primarily along the A-P direction. The possibility that epidermis could provide an asymmetric driving force for tail-bending can be ruled out through proof by contradiction. If the epidermis division provides a part of the asymmetric driving force for tail-bending, the epidermal cells will be in a compressive state along the A-P direction, as shown in Fig. 6F. Due to the Poisson's effect, the epidermal cells along the D-V axis should become higher as the tail bends. The deduction that the height of the epidermal cells will increase is contrary to our experimental observations (Table S1). Therefore, the assumption that the epidermis division provides a part of the asymmetric driving force for tail bending is unreasonable. In this way, we can rule out the possibility that epidermis could provide a part of the asymmetric driving force for tail-bending. In addition, the stress state of the notochord is more complex than the epidermal cells, so the changes of the cell height cannot be used to infer the stress state.
As we have shown in the main text, both contraction of the notochord and asymmetric division of the epidermis could lead to the bending of the tail. However, our finite element simulation shows that the stress state and shape change of the epidermis are strikingly different under these two scenarios. Especially, if the asymmetric division plays the main role, the epidermal cells will be in a compressive state along the anterior-posterior direction, as shown in Fig. 6F. Because of the Poisson's effect, the height of the epidermal cells along DV axis should be larger as the tail bends.

(8) More generally, if the finite element simulations do not explicitly capture the combination of tail extension and bending, I do not see how their predictions can be compared in a meaningful way to the experimental observations/measurements. To be clear, I support the use of modeling to help interpret these data. I just think the authors need to be more clear about the modeling assumptions, if they have not done so, they need to consider simultaneously the dynamics of tail extension and bending. In fact, I think that doing this more explicitly would provide a richer context in which to interpret e.g. the perturbation experiments shown in Figure 4.

Response: The beam extends and bends simultaneously. During the tail bending, the movement of the notochord can be treated as the superposition of the elongation and asymmetric contraction. Therefore, the equivalent movement of the notochord is an asymmetric elongation: the dorsal notochord elongates more than the ventral notochord. This equivalent movement has been considered in our model, namely, the combination of tail extension and bending has been captured.

(9) I think there are some simple ways in which the authors could further assess the relative magnitudes and autonomy of contributions of notochord and epidermis to tail bending that do not rely on indirect inference from mechanical models. For example, if the authors prevent notochord extension (e.g. by inhibiting prickle or disheveled), do the asymmetries in epidermal cell divisions persist? In the aphidicolin (or cdc-45) experiments, what happens to the asymmetries in F-actin and Myosin II in the notochord? Do they persist? When the tail bends in the opposite direction, is this associated with a change in F-actin and Myosin II distributions? If not, then doesn't this imply that (at least in this perturbed condition) that actomyosin asymmetries are not determining the direction of tail bending?

Response: We checked the mutant images of *Aim* (prickle) (Jiang et al., 2015) and *chm* (laminin) (Veeman et al., 2018) and found that there is no apparent dorsal asymmetry of epidermis cells and no tail-bending either in these mutants. In our inhibitor treatment and the overexpression of cdc45

experiments, we did not observe the clear ventral-polarized actin in notochord. In those dorsally-bent embryos, we did not observe the dorsal-polarized actomyosin in notochord either.
We speculated that these observations could neither provide us the support evidence, nor rule against our conclusion. Because, all those dorsal-bending happened at later stage. They showed normally ventral-bending at early stage (Fig. 4H), indicating that when the dorsal cell division was disrupted, the driving mechanisms for ventral-bending at early stage and dorsal-bending at later stage are different. We speculated that at later stage, the stretch generated from notochord elongation drove the tail-bending dorsally, since the cell division was disrupted at dorsal epidermis.

(10) The paper is full of awkward phrasing, misspelled and misused words. All of this could be easily solved by asking a native English-speaking colleague for editorial input.

Response: The English writing of manuscript has been checked and edited carefully by a native English speaker.

References

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Second decision letter

MS ID#: DEVELOP/2019/185868

MS TITLE: Ciona embryonic tail bending is driven by asymmetrical notochord contractility and epithelial proliferation

AUTHORS: Qiongxuan LU, Yuan Gao, Yuanyuan Fu, Hongzhe Peng, Wenjie Shi, Bo Li, Xi-Qiao Feng, and Bo Dong

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 overal quite positive but you need to address the few remaining comments form the reviewers, especially comment 2 from Reviewer 2 regarding the modelling so we may consider your manuscript further. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response.

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.
HPoint 1 from Rev 2 requires an additional experiment. ALl other comments require rewriting, detailed explanation and can be done without proper access to the lab.

Reviewer 1

Advance summary and potential significance to field

The main interest of this manuscript is to provide a mechanical understanding of tail bending among chordate embryos.

Comments for the author

Their revised manuscript offers a significant improvement over the original version, but in my opinion the English remains poor. What about contacting a professional publishing office to improve it further.

Regarding the data, I was convinced by the added experiences or clarifications. Having said that, it would be important to underline in the text (lines 175-183) that the ventral phospho-rMLC enrichment is tiny (1.3 times). Regarding the model, I would like a better description of it, and a more precise reference to the value of the constriction force taken from the amnioserosa because the reference is a book chapter that is not freely available online.

Finally, the title emphasizes the role of both the contribution of asymmetrical notochordal contractility and epithelial proliferation, which is indeed supported by Figures 2-4. However, Figure 5 suggests that asymmetric notochord contractility may be sufficient to achieve flexion. Hence, the title could be modified to reflect this. In addition, I regret that the authors did not attempt in their modeling to really account for the respective contributions of notochord contractility versus epithelial proliferation to tail bending.

Reviewer 2

Advance summary and potential significance to field

See previous review. My overall assessment about the advance and potential significance remains the same

Comments for the author

This revised manuscript is responsive to many of my previous comments and concerns. I remain positive overall, but there are a two outstanding issues that I remain concerned about.

(1) The cofilin experiments

In response to my previous comments, the authors have provided more background information and added a few citations suggesting that a overexpression of a non-phosphorylatable mutant of cofilin could act as a dominant negative to suppress Rho-mediated actin polymerization. However, their claim that overexpression of the mutant cofilin disrupts the ventral F-actin enrichment remains completely unsupported. I don't see why they could not simply fix embryos overexpressing the mutant cofilin and stain with phalloidin to verify that the cofilin mutant suppresses actin assembly (and specifically the ventral enrichment of F-actin if that is what they want to claim). Without such a simple control, it is difficult to attach any real weight to these observations.

(2) The computational modeling

The authors have not addressed my primary concern about the computational modeling which is that they have not provided enough information about how the simulations were done for a reader to draw any meaningful conclusions. How do they impose the active forces in the "epidermis driving" vs notochord driving" scenarios? What is the distribution and magnitude of these forces? How do they incorporate the effects of active cell division into this model?
Are they evaluating extension vs compression with respect to some fixed reference state? If so, why is this a reasonable assumption? Embryonic cells are not simple elastic materials - they can set a preferred shape through a dynamic balance of forces (e.g. contractile forces) that can change over time.

Without knowing more about how they implemented the model, I cannot evaluate the claims they make in the manuscript, or the arguments they have made in response to my previous comments

I am not necessarily saying that the authors have to change their model, but they do need to provide enough information about the assumptions and implementation for an intelligent reader to evaluate their conclusions.

Second revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field: Reviewer 1 Advance Summary and Potential Significance to Field: The main interest of this manuscript is to provide a mechanical understanding of tail bending among chordate embryos.
Reviewer 1 Comments for the Author:

Their revised manuscript offers a significant improvement over the original version, but in my opinion the English remains poor. What about contacting a professional publishing office to improve it further.

Response: We thank the reviewer for the positive evaluation. We have now used Springer Nature Language Editing service to proofread our manuscript.

Regarding the data, I was convinced by the added experiences or clarifications. Having said that, it would be important to underline in the text (lines 175-183) that the ventral phospho-rMLC enrichment is tiny (1.3 times).

Response: Thanks for these comments. As for the description of phospho-rMLC enrichment, we have revised the text to 'We examined active myosin II… and found that it asymmetrically accumulated at the notochord's ventral side during embryonic tail bending (Fig. 2D, yellow arrows, $n \geq 6$). Quantitative data further revealed that the difference was 1.3 times, relatively weaker compared to F-actin (Fig. 2F).' (Page 6, line 161-165)

Regarding the model, I would like a better description of it, and a more precise reference to the value of the constriction force taken from the amnioserosa because the reference is a book chapter

that is not freely available online.

Response: We thank the referee for the beneficial suggestions. We rewrite the biomechanical model to illustrate the underlying mechanisms more clearly (Page 9-12, line 241-312). A theoretical physical model is added in the revised supplemental material to quantitatively evaluate the contribution of the epidermis to the tail bending. A more precise value for the constriction force is adopted based on different experiments (Effler et al., 2006; Poirier et al., 2012; Soine et al., 2015; Zhang and Robinson, 2005). In our study, we took 10 nN as the value of constriction force in our modeling. We have added these references in our revised manuscript (Page 11, line 307-308).

Finally, the title emphasizes the role of both the contribution of asymmetrical notochordal contractility and epithelial proliferation, which is indeed supported by Figures 2-4. However, Figure 5 suggests that asymmetric notochord contractility may be sufficient to achieve flexion. Hence, the title could be modified to reflect this.

Response: Thanks for the suggestion. The title has been changed as "*Ciona* embryonic tail bending is driven by asymmetrical notochord contractility and coordinated by epithelial proliferation".

In addition, I regret that the authors did not attempt in their modeling to really account for the respective contributions of notochord contractility versus epithelial proliferation to tail bending.

Response: We thank the referee for the beneficial suggestions. In the revised manuscript, we add a theoretical model to quantitatively evaluate the contribution of the epithelial proliferation and the results show that the bending moment caused by epithelial proliferation is relatively tiny compared to the total moment required to bend the tail. However, the underlying mechanisms of notochord contractility is certain complex. To account for this process, one need to know the mechanical properties and kinematics of the actin and myosin at the ventral and dorsal sides of the notochord, which is a huge challenge for experiments. Therefore, an indirect method is adopted. The total moment required to bend the tail and the bending moment induced by the epithelial proliferation can be calculated based on the geometric parameters of the embryos for different stages. Then the bending moment induced by the notochord contractility can be obtained by subtraction. To further confirm the conclusion, we calculate the bending angle caused by the notochord contractility using the reference value of contractile force from similar experiments (Effler et al., 2006; Poirier et al., 2012; Soine et al., 2015; Zhang and Robinson, 2005), which shows that the notochord contractility is sufficient to bend the tail to a certain degree. Taking all the results into consideration, we conclude that notochord contractility is the main driving force for tail bending, while epithelial proliferation is dispensable.

Reviewer 2 Advance Summary and Potential Significance to Field:

See previous review. My overall assessment about the advance and potential significance remains the same

Reviewer 2 Comments for the Author:

This revised manuscript is responsive to many of my previous comments and concerns. I remain positive overall, but there are two outstanding issues that I remain concerned about.

Response: We appreciate the reviewer for the positive comments.

(1) The cofilin experiments

In response to my previous comments, the authors have provided more background information and added a few citations suggesting that a overexpression of a non- phosphorylatable mutant of cofilin could act as a dominant negative to suppress Rho- mediated actin polymerization. However, their claim that overexpression of the mutant cofilin disrupts the ventral F-actin enrichment remains completely unsupported. I don't see why they could not simply fix embryos overexpressing the mutant cofilin and stain with phalloidin to verify that the cofilin mutant suppresses actin assembly (and specifically the ventral enrichment of F-actin if that is what they want to claim). Without such a simple control, it is difficult to attach any real weight to these observations.

Response: We thank the reviewer for this reminding. According to the suggestion, we carried out additional experiment to examine the effects of the mutant cofilin on the ventral F-actin enrichment. The results showed that the ventral F-actin enrichment was diminished in cofilin S5A mutant overexpressing notochord compared to the control ones (new Fig. 3C), suggesting that the dynamic actin polymerization mediated by functional cofilin is required for the notochord's ventral F-actin enrichment. We have now updated Fig.3 and added the corresponding description 'Cofilin S5A mutant overexpression decreased the ventral asymmetrical polarized F-actin in the cofilin S5A-expressing notochord (n = 16/24; Fig. 3C, top) compared to the control group (n = 15/19; Fig. 3C, bottom).' in the text (Page 7-8, line 196-199).

(2) The computational modeling

The authors have not addressed my primary concern about the computational modeling, which is that they have not provided enough information about how the simulations were done for a reader to draw any meaningful conclusions.

Response: We thank the referee for the beneficial suggestions. We rewrite the modeling process in detail to illustrate the underlying mechanisms more clearly (Page 9-10, lines 253–260). During the tail bending, the parameters we can obtain from experiments are merely the geometric parameters of the tail and the Young's moduli of different types of cells. The forces induced by epithelial proliferation and notochord contractility during tail bending are hard to be obtained from experiments. Therefore, the finite element model is somewhat a qualitative model. Using this model, we can simulate the deformation process of the tail bending and the stress state (compressive or tensile) is straightforward.

How do they impose the active forces in the "epidermis driving" vs notochord driving" scenarios? What is the distribution and magnitude of these forces?

Response: In the finite element simulations, we considered two extreme situations: the contraction of the notochord serves as the exclusive driving force, and the differential cell division serves as the exclusive driving force. For the notochord driving scenario, the elongation and contraction of the notochord are considered together and included by a gradient growth field, while the epidermal cells are treated as a passive material, undergoing elastic deformation. For the differential cell division driving scenario, the differential cell division is included by a gradient growth field, while the notochord is treated as a passive material. In these simulations, the magnitudes of the forces do not agree with the real forces in the tail. However, based on the stress state of the tail (compressive or tensile) and the changes of geometric parameters of the tail, we can exclude the possibility that cell division serves as the main driving force for tail bending.

Just as pointed by the referee in the previous review: *"In principle, tail bending could be explained by superimposing upon this active extension force either a difference in actomyosin contractility along the notochord boundary, or a dorsal vs ventral asymmetry in active spreading of the epidermis. Importantly, both scenarios could lead to the observed decreased in height of epidermal cells, depending on the rates and patterns of cell divisions."* The qualitative analysis cannot exclude the situation that the epithelial proliferation drives tail bending up to a point. Therefore, we add a theoretical model in the supplemental material. The differential proliferation of the epidermis is included in the model by the volumetric growth theory. Specifically, the differential proliferation of the epidermis is implemented by a gradient growth strain as shown in Fig. S4A. Based on the geometric parameters of the tail for different stages, we can obtain the bending moment induced by epithelial proliferation and the total moment required to bend the tail. The results show that the bending moment induced by the epidermis is dispensable compared to the moment required to bend the tail. Therefore, the contribution of the epidermis differential cell division to the tail bending at the initial stage is relatively limited. To further confirm the conclusion, we calculate the bending angle caused by the notochord contractility using the reference value of contractile force from similar experiments (Effler et al., 2006; Poirier et al., 2012; Soine et al., 2015; Zhang and Robinson, 2005), which shows that the notochord contractility is sufficient to bend the tail to a certain degree. Taking all the results into consideration, we conclude that notochord contractility is the main driving force for tail bending, while epithelial proliferation is dispensable.

How do they incorporate the effects of active cell division into this model? Are they evaluating extension vs compression with respect to some fixed reference state? If so, why is this a reasonable assumption?

Response: In the finite element simulations, the cell division is included by a gradient growth, which can be implemented similar to thermal expansion (Page 9-10, lines 253– 260). Under this growth field, the dorsal side of the epidermis will be elongated more than the ventral side. In the added theoretical model, the possible two kind of effects of active cell division are discussed (Supplemental Information) and the contributions of the epithelial proliferation under these two cases are calculated, as shown in Figs. S4C and S4D. The effects of differential cell divisions may be divided into two portions: cell divisions may enlarge the total volume of the epidermis and further bend the tail; and cell divisions along the A-P axis may actively elongate the cells along the A-P axis, shortening the cell width correspondingly.

Strictly speaking, the absolute extension or compression states of the cells are hard to identify just from the geometric parameters. In our analysis, we assume that at the beginning of the tail bending (e.g., tailbud stage 17) the tail state is the fixed reference state. This assumption may introduce some deviations for the absolute value of forces but the relative sizes are conserved.

Embryonic cells are not simple elastic materials - they can set a preferred shape through a dynamic balance of forces (e.g. contractile forces) that can change over time. Without knowing more about how they implemented the model, I cannot evaluate the claims they make in the manuscript, or the arguments they have made in response to my previous comments.

I am not necessarily saying that the authors have to change their model, but they do need to provide enough information about the assumptions and implementation for an intelligent reader to evaluate their conclusions.

Response: We agree that embryonic cells are not simple elastic materials. A tissue deforms elastically if it is deformed on short time scales (less than a few minutes); cells change their geometry. On longer time scales (tens of minutes), cells change their position by remodeling the topology of cell contacts and thereby dissipate the stress like a viscous fluid (Guillot and Lecuit, 2003). During the tail bending, the total time of the tail bending is pretty long (maybe several hours), however, the actual deformation process of the tail is certain short and thus the tail bending can be treated as a series of elastic deformation processes. Further, this simplification may influence the absolute magnitude of the contributions, while the relative contributions are conserved.

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Third decision letter

MS ID#: DEVELOP/2019/185868

MS TITLE: Ciona embryonic tail bending is driven by asymmetrical notochord contractility and coordinated by epithelial proliferation

AUTHORS: Qiongxuan LU, Yuan Gao, Yuanyuan Fu, Hongzhe Peng, Wenjie Shi, Bo Li, Zhiyi Lv, Xi-Qiao Feng, and Bo Dong

I must sincerely apologise for the very long delay before being able to come back to you. The current epidemics complicates the review process. I have now received the comments from one of the reviewers who was the most critical about your 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.

This referee appreciates the improvement of your manuscript but does require further clarification in the text before we can go ahead with publication of your revised manuscript. Please attend to these comments in your revised manuscript and detail them in your point-by-point response.

Reviewer 2

Advance summary and potential significance to field

I've had a look at the revised manuscript. They have been responsive to my previous concerns, and the manuscript is certainly improved. I think I now understand what they have done with the modeling and with a few small caveats
(see below), I think their conclusions are reasonable. I have only a few points that they could quickly address.

Comments for the author

(1) The new data on F-actin staining in the cofilin mutants is an important addition, but it is presented in a confusing way. In the text, they seem to be claiming that overexpressing the DN Cofilin decreases ventral F-actin.
The micrographs of phalloidin-stained embryos show clearly that the difference btw ventral and dorsal F-actin enrichment is greatly reduced, but the two micrographs showing phalloidin-staining in control and DN-Cofilin expressing embryo appear to have been processed differently.

Maybe they only mean to say that the difference between dorsal and ventral is reduced. If so, they should clarify this in the main text. If they want to make a claim about an absolute change in levels, then they need to show a quantitative comparison of measured phalloidin staining intensities, and they should show a comparison of micrographs that were treated identically

(2) On lines 262-4, it would be helpful if they could add a sentence to clarify for the reader what it means to impose a gradient growth field. In particular, what sets the magnitude of the force produced by the gradient growth field and how is this determined? In the notochord exclusive scenario, do they adjust the magnitude to produce the observed degree of extension bending (in the notochord exclusive scenario) and the observed degree of bending (in the epidermis scenario) or something else.? This should be clearly stated so the subsequent arguments on lines 265-277 can be understood.

(3) I still find the argument on lines 265-277 hard to parse. They seem to be saying: Imposing a growth gradient field on the epidermis alone that is sufficient to cause the observed bending induces compression of the epidermal cells, and because compression is not observed in embryos, cell division cannot explain the bending. But this is an unfair comparison because in the epidermis scenario alone, the stiffer notochord will resist extension, whereas the notochord is undergoing active extension in the embryo. A fairer test would be one in which the dorsal/ventral bias on epidermal cell division is imposed on active (symmetric) notochord extension.

I am not suggesting that they should do new simulations, but I think the text/conclusions on lines 265-277 should acknowledge the caveat that the epidermis only scenario ignores the contribution of active notochord extension.

Third revision

Author response to reviewers' comments

Reviewer 2 Advance Summary and Potential Significance to Field:
I've had a look at the revised manuscript. They have been responsive to my previous concerns, and the manuscript is certainly improved. I think I now understand what they have done with the modeling and with a few small caveats (see below), I think their conclusions are reasonable. I have only a few points that they could quickly address.

Response: Thanks. All your comments are indeed helpful for the improvement of our manuscript.

Reviewer 2 Comments for the Author:
(1) The new data on F-actin staining in the cofilin mutants is an important addition, but it is presented in a confusing way. In the text, they seem to be claiming that overexpressing the DN Cofilin decreases ventral F-actin. The micrographs of phalloidin-stained embryos show clearly that the difference btw ventral and dorsal F-actin enrichment is greatly reduced, but the two micrographs showing phalloidin-staining in control and DN-Cofilin expressing embryo appear to have been processed differently.
Maybe they only mean to say that the difference between dorsal and ventral is reduced. If so, they should clarify this in the main text. If they want to make a claim about an absolute change in levels, then they need to show a quantitative comparison of measured phalloidin staining intensities, and they should show a comparison of micrographs that were treated identically

Response: The phalloidin-staining images of control and DN-Cofilin are made from the originally obtained confocal pictures. Indeed, they look different. But we don't compare the actin intensity between control and DN-cofilin embryos. Here, as the reviewer pointed out, we only want to show that DN-Cofilin decreased the difference between ventral and dorsal F-actin enrichment in DN-Cofilin overexpressing embryos. Therefore, we have clarified this point in the text (Line 196-198) in the revised version.

(2) On lines 262-4, it would be helpful if they could add a sentence to clarify for the reader what it means to impose a gradient growth field. In particular, what sets the magnitude of the force produced by the gradient growth field and how is this determined? In the notochord exclusive scenario, do they adjust the magnitude to produce the observed degree of extension bending (in the notochord exclusive scenario) and the observed degree of bending (in the epidermis scenario) or something else.? This should be clearly stated so the subsequent arguments on lines 265-277 can be understood.

Response: We thank the referee for the beneficial suggestions. We had added more details about the growth field (Lines 256–261). When we impose a growth field, we actually set a positive or negative growth ratio on the tissues. Tissues with a positive growth ratio will enlarge, while tissues with a negative growth ratio will shrink. In this vein, a growth field can be utilized to simulate the tissue elongation or contraction. To simulate the bending of the tail, a simple and typical method is to impose a gradient growth field, as shown in Fig. S4A.

In the simulation, we can adjust the magnitude of the growth field to fit the bending angle and the elongation of the tail observed in the experiments. However, the stress or strain level in the tail does not influence the relative contributions of the epidermis and notochord. We have explained this in Lines 266–268 in the revised version.

(3) I still find the argument on lines 265-277 hard to parse. They seem to be saying: Imposing a growth gradient field on the epidermis alone that is sufficient to cause the observed bending induces compression of the epidermal cells, and because compression is not observed in embryos, cell division cannot explain the bending. But this is an unfair comparison, because in the epidermis scenario alone, the stiffer notochord will resist extension, whereas the notochord is undergoing active extension in the embryo. A fairer test would be one in which the dorsal/ventral bias on epidermal cell division is imposed on active (symmetric) notochord extension.

I am not suggesting that they should do new simulations, but I think the text/conclusions on lines 265-277 should acknowledge the caveat that the epidermis only scenario ignores the contribution of active notochord extension.

Response: We thank the referee for the beneficial suggestions. We agree with the reviewer that the active notochord extension plays an important role. The extension of the notochord will stretch the epidermal cells and counteract the compression of the epidermal cells to some extent. Therefore, based on the simulation results merely we cannot totally exclude the possibility that the differential cell division can drive the tail bending. Actually, the magnitude of the notochord extension is hard to obtain from experiments. Hence, the epidermis only scenario we adopt in the manuscript is just an extreme case. We make up this caveat and quantify the contributions of the epidermis and notochord by means of a theoretical model (see Supplemental Information). In the theoretical model, we calculated the contributions of the epidermis and notochord based on the geometrical parameters at different stages. Differential epidermal cell division, active (symmetric) notochord extension, and notochord contraction are all considered. Based on the finite element simulations and the theoretical model, we can arrive at the final conclusion that asymmetrical notochord actomyosin constriction plays a dominant role in the tail bending.
In response to these comments, we have acknowledged the caveat and modified the corresponding description (Lines 279–283) in the revised manuscript.

Fourth decision letter

MS ID#: DEVELOP/2019/185868

MS TITLE: Ciona embryonic tail bending is driven by asymmetrical notochord contractility and coordinated by epithelial proliferation

AUTHORS: Qiongxuan LU, Yuan Gao, Yuanyuan Fu, Hongzhe Peng, Wenjie Shi, Bo Li, Zhiyi Lv, Xi-Qiao Feng, and Bo Dong
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.