JCB

Architecture of the cortical actomyosin network driving apical constriction in C. elegans

Pu Zhang, Taylor Medwig-Kinney, and Bob Goldstein

Corresponding Author(s): Bob Goldstein, University of North Carolina at Chapel Hill

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April 6, 2023

Re: JCB manuscript #202302102

Dr. Bob Goldstein
University of North Carolina at Chapel Hill
Department of Biology
Chapel Hill, North Carolina 27599-3280

Dear Dr. Goldstein,

Thank you for submitting your manuscript entitled "Architecture of the cortical actomyosin network driving apical constriction in C. elegans." The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revision if you can address the reviewers' key concerns, as outlined here.

You will see that the reviewers feel the study is suitable for the JCB Report format but request additional experiments to clarify the distributions of F-actin and UNC-94.

While you are revising your manuscript, please also attend to the following editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

GENERAL GUIDELINES:
Text limits: Character count for a Report is < 20,000, not including spaces. Count includes title page, abstract, introduction, the joint Results & Discussion, and acknowledgments. Count does not include materials and methods, figure legends, references, tables, or supplemental legends.

Figures: Reports may have up to 5 main text figures. To avoid delays in production, figures must be prepared according to the policies outlined in our Instructions to Authors, under Data Presentation, https://jcb.rupress.org/site/misc/ifora.xhtml. All figures in accepted manuscripts will be screened prior to publication.

IMPORTANT: It is JCB policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Supplemental information: There are strict limits on the allowable amount of supplemental data. Reports may have up to 3 supplemental figures. Up to 10 supplemental videos or flash animations are allowed. A summary of all supplemental material should appear at the end of the Materials and methods section.

Please note that JCB now requires authors to submit Source Data used to generate figures containing gels and Western blots with all revised manuscripts. This Source Data consists of fully uncropped and unprocessed images for each gel/blot displayed in the main and supplemental figures. If the revised paper will contain cropped gel and/or blot images, please be sure to provide one Source Data file for each figure that contains gels and/or blots along with your revised manuscript files. File names for Source Data figures should be alphanumeric without any spaces or special characters (i.e., SourceDataF#, where F# refers to the associated main figure number or SourceDataFS# for those associated with Supplementary figures). The lanes of the gels/blots should be labeled as they are in the associated figure, the place where cropping was applied should be marked (with a box), and molecular weight/size standards should be labeled wherever possible. Source Data files will be made available to reviewers during evaluation of revised manuscripts and, if your paper is eventually published in JCB, the files will be directly linked to specific figures in the published article.
Source Data Figures should be provided as individual PDF files (one file per figure). Authors should endeavor to retain a minimum resolution of 300 dpi or pixels per inch. Please review our instructions for export from Photoshop, Illustrator, and PowerPoint here: https://rupress.org/jcb/pages/submission-guidelines#revised

The typical timeframe for revisions is three to four months. While most universities and institutes have reopened labs and allowed researchers to begin working at nearly pre-pandemic levels, we at JCB realize that the lingering effects of the COVID-19 pandemic may still be impacting some aspects of your work, including the acquisition of equipment and reagents. Therefore, if you anticipate any difficulties in meeting this aforementioned revision time limit, please contact us and we can work with you to find an appropriate time frame for resubmission. Please note that papers are generally considered through only one revision cycle, so any revised manuscript will likely be either accepted or rejected.

When submitting the revision, please include a cover letter addressing the reviewers' comments point by point. Please also highlight all changes in the text of the manuscript.

We hope that the comments below will prove constructive as your work progresses. We would be happy to discuss them further once you've had a chance to consider the points raised in this letter.

Thank you for this interesting contribution to Journal of Cell Biology. You can contact us at the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Sincerely,

William Bement, PhD
Monitoring Editor
Journal of Cell Biology

Dan Simon, PhD
Scientific Editor
Journal of Cell Biology

Reviewer #1 (Comments to the Authors (Required)):

In this work the authors provide new insights into apical constriction of endodermal precursor cells during C. elegans gastrulation. They show that the organization of actin, non-muscle myosin-2 and myosin-activating kinase MRCK-1 is consistent with a diffusely organized medioapical actomyosin network which drives apical constriction. Apical constriction occurs during morphogenesis in many different contexts with different actomyosin network organizations and this work makes an important contribution to this area and is likely to be of broad interest to the field and the readership of JCB.

The data is of high-quality and uses endogenously tagged proteins to determine the actomyosin organization in endoderm precursor cells during internalization. The distribution of UNC-94 neon green, used to highlight pointed ends of actin filaments, is more challenging to see in the images shown, though the line scans are consistent with the author's conclusions. Would it be possible to confirm this either with a second marker or by staining fixed samples with an antibody to neon Green to amplify the signal?

Reviewer #2 (Comments to the Authors (Required)):

This paper provides an interesting contrast (distributed and non-polarized network) to two other modes of apical constriction (purse string and central myosin). This is a nice, focused paper with a clearly described result. Although the work is descriptive, it is quantitative and beautifully executed with lovely imaging. I think it will be widely viewed and cited by those interested in actomyosin contractility and mechanisms of development.

Table S1 is a nice reference for the phenotypic variation seen across different species.

I really have very little to add; in my opinion this piece is ready to publish essentially as written.

Minor:
Table S3, please give N
The JCB review software says 'no funding source' but the acknowledgements say the work was supported by an NIH R35.

Reviewer #3 (Comments to the Authors (Required)):

Zhang et al show that actin filaments and non-muscle myosin are not distributed in a radial array, with myosin in the center and filaments projecting outward, within the C. elegans gastrulating and apically constricting endodermal precursors, in contrast to ventral closure in Drosophila. They do this by identifying likely plus and minus end binding proteins and documenting their distributions, along with non muscle myosin and one myosin regulator.

Some minor comments for the authors consideration:

1. Are there any phenotypes associated with knockdown of the end binding proteins?

2. Can the authors document the distribution of actin filaments in the apical surfaces, with two color imaging to show the

relationship between end binding proteins and microfilaments? Perhaps such live imaging would provide insight into mechanism.

3. The authors should provide the number of embryos scored for documenting hatching rates in the transgenic lines used.

1st Revision - Authors' Response to Reviewers: May 24, 2023

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

Bob Goldstein
James L. Peacock III Distinguished Professor
Department of Biology

CB# 3280, 616 Fordham Hall
University of North Carolina, Chapel Hill
Chapel Hill, NC 27599

May 24, 2023

Re: JCB manuscript #202302102

Dear Dr. William Bement and Dr. Dan Simon,

We are pleased to resubmit our revised manuscript entitled, “Architecture of the cortical actomyosin network driving apical constriction in *C. elegans*.” We made the following changes in response to reviewer feedback:

- We imaged the tagged UNC-94 allele with optimized imaging parameters to achieve a better signal-to-noise ratio of pointed-end capping protein localization, which was consistent with what we had already observed and quantified.
- We generated a single-copy transgene to label actin (LifeAct::mScarlet-I), which we paired with the tagged capping protein alleles to visualize their co-localization.
- We added sample size information for the embryo hatching assays.

These new pieces of data have been added to the manuscript (text additions highlighted in red) and are outlined below in our point-by-point response to reviewer feedback.

Reviewer #1 (Comments to the Authors (Required)):

In this work the authors provide new insights into apical constriction of endodermal precursor cells during C. elegans gastrulation. They show that the organization of actin, non-muscle myosin-2 and myosin-activating kinase MRCK-1 is consistent with a diffusely organized medioapical actomyosin network which drives apical constriction. Apical constriction occurs during morphogenesis in many different contexts with different actomyosin network organizations and this work makes an important contribution to this area and is likely to be of broad interest to the field and the readership of JCB.

We thank the reviewer for recognizing the importance of our work in contributing toward the broader goal of comparing actomyosin organization in different biological contexts.

The data is of high-quality and uses endogenously tagged proteins to determine the actomyosin organization in endoderm precursor cells during internalization. The distribution of UNC-94 neon green, used to highlight pointed ends of actin filaments, is more challenging to see in the images shown, though the line scans are consistent with the author's conclusions. Would it be possible to confirm this either with a second marker or by staining fixed samples with an antibody to neon Green to amplify the signal?

For all of our time lapse imaging, we selected imaging conditions to balance optimization of signal intensity and minimization of photobleaching. Following this reviewer's suggestion, we performed static imaging of UNC-94::mNeonGreen using a higher laser power and longer exposure time, and added a representative image as panel D of Figure S3. The localization pattern was consistent with what we had previously observed and quantified (Figure 4D,F).

Reviewer #2 (Comments to the Authors (Required)):

This paper provides an interesting contrast (distributed and non-polarized network) to two other modes of apical constriction (purse string and central myosin). This is a nice, focused paper with a clearly described result. Although the work is descriptive, it is quantitative and beautifully executed with lovely imaging. I think it will be widely viewed and cited by those interested in actomyosin contractility and mechanisms of development.

Table S1 is a nice reference for the phenotypic variation seen across different species.

I really have very little to add; in my opinion this piece is ready to publish essentially as written.

We thank the reviewer for this positive feedback on the quality of our imaging and how the manuscript was written.

Minor:
Table S3, please give N

The sample size for each embryo hatching rate experiment has been added to Table S3.

The JCB review software says 'no funding source' but the acknowledgements say the work was supported by an NIH R35.

Thank you for bringing this to our attention. We will ensure that our funding sources are accurately reflected in the published manuscript.

Reviewer #3 (Comments to the Authors (Required)):

Zhang et al show that actin filaments and non-muscle myosin are not distributed in a radial array, with myosin in the center and filaments projecting outward, within the C. elegans gastrulating and apically constricting endodermal precursors, in contrast to ventral closure in Drosophila. They do this by identifying likely plus and minus end binding proteins and documenting their distributions, along with non muscle myosin and one myosin regulator.

Some minor comments for the authors consideration:

1. Are there any phenotypes associated with knockdown of the end binding proteins?

We agree that it would be interesting to disrupt these proteins' functions and examine phenotypes in detail to determine the functional consequence of perturbing capping proteins.

However, the conclusions we are reaching regarding actomyosin organization do not depend on this. Therefore we consider this beyond the scope of the manuscript.

> *2. Can the authors document the distribution of actin filaments in the apical surfaces, with two color imaging to show the relationship between end binding proteins and microfilaments? Perhaps such live imaging would provide insight into mechanism.*

Because the F-actin label does not provide filament polarity information, we did not expect it to be valuable as an indicator of actomyosin network organization. Still, to visualize F-actin together with the two capping proteins, we generated a single-copy transgene to label F-actin (*mex-5p*::LifeAct::mScarlet-I), re-tagged CAP-1 with mTurquoise2, and combined these with mNeonGreen-tagged UNC-94 in a triple-labeled strain. This allowed us to visualize localization of actin with that of barbed- and pointed-end binding proteins, which is now added as panel E of Figure S3. This strain's utility for quantification of protein localization was limited due to signal bleed-through between channels, so we stuck with our double-labeled strain for quantification.

> *3. The authors should provide the number of embryos scored for documenting hatching rates in the transgenic lines used.*

The sample size for each embryo hatching rate experiment has been added to Table S3.

Sincerely,

Bob Goldstein Pu Zhang Taylor Medwig-Kinney

May 30, 2023

RE: JCB Manuscript #202302102R

Dr. Bob Goldstein
University of North Carolina at Chapel Hill
Department of Biology
Chapel Hill, North Carolina 27599-3280

Dear Dr. Goldstein,

Thank you for submitting your revised manuscript entitled "Architecture of the cortical actomyosin network driving apical constriction in C. elegans." We would be happy to publish your paper in JCB pending final revisions necessary to meet our formatting guidelines (see details below).

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://jcb.rupress.org/submission-guidelines#revised. **Submission of a paper that does not conform to JCB guidelines will delay the acceptance of your manuscript.**

1) Text limits: Character count for Reports is < 20,000, not including spaces. Count includes title page, abstract, introduction, results & discussion, and acknowledgments. Count does not include materials and methods, figure legends, references, tables, or supplemental legends.

2) Figure formatting: Reports may have up to 5 main text figures. Scale bars must be present on all microscopy images, including inset magnifications. Please avoid pairing red and green for images and graphs to ensure legibility for color-blind readers. If red and green are paired for images, please ensure that the particular red and green hues used in micrographs are distinctive with any of the colorblind types. If not, please modify colors accordingly or provide separate images of the individual channels.

3) Statistical analysis: Error bars on graphic representations of numerical data must be clearly described in the figure legend. The number of independent data points (n) represented in a graph must be indicated in the legend. Please, indicate whether 'n' refers to technical or biological replicates (i.e. number of analyzed cells, samples or animals, number of independent experiments). If independent experiments with multiple biological replicates have been performed, we recommend using distribution-reproducibility SuperPlots (please see Lord et al., JCB 2020) to better display the distribution of the entire dataset, and report statistics (such as means, error bars, and P values) that address the reproducibility of the findings.

Statistical methods should be explained in full in the materials and methods. For figures presenting pooled data the statistical measure should be defined in the figure legends. Please also be sure to indicate the statistical tests used in each of your experiments (both in the figure legend itself and in a separate methods section) as well as the parameters of the test (for example, if you ran a t-test, please indicate if it was one- or two-sided, etc.). Also, if you used parametric tests, please indicate if the data distribution was tested for normality (and if so, how). If not, you must state something to the effect that "Data distribution was assumed to be normal but this was not formally tested."

4) Materials and methods: Should be comprehensive and not simply reference a previous publication for details on how an experiment was performed. Please provide full descriptions (at least in brief) in the text for readers who may not have access to referenced manuscripts. The text should not refer to methods "...as previously described."

5) For all cell lines, vectors, constructs/cDNAs, etc. - all genetic material: please include database / vendor ID (e.g., Addgene, ATCC, etc.) or if unavailable, please briefly describe their basic genetic features, even if described in other published work or gifted to you by other investigators (and provide references where appropriate). Please be sure to provide the sequences for all of your oligos: primers, si/shRNA, RNAi, gRNAs, etc. in the materials and methods. You must also indicate in the methods the source, species, and catalog numbers/vendor identifiers (where appropriate) for all of your antibodies, including secondary. If antibodies are not commercial, please add a reference citation if possible.

6) Microscope image acquisition: The following information must be provided about the acquisition and processing of images:
a. Make and model of microscope
b. Type, magnification, and numerical aperture of the objective lenses
c. Temperature

d. Imaging medium
e. Fluorochromes
f. Camera make and model
g. Acquisition software
h. Any software used for image processing subsequent to data acquisition. Please include details and types of operations involved (e.g., type of deconvolution, 3D reconstitutions, surface or volume rendering, gamma adjustments, etc.).

7) References: There is no limit to the number of references cited in a manuscript. References should be cited parenthetically in the text by author and year of publication. Abbreviate the names of journals according to PubMed.

8) Supplemental materials: There are strict limits on the allowable amount of supplemental data. Articles/Tools may have up to 5 supplemental figures and 10 videos. Please also note that tables, like figures, should be provided as individual, editable files. A summary of all supplemental material should appear at the end of the Materials and methods section. Please include one brief sentence per item.

9) eTOC summary: A ~40-50 word summary that describes the context and significance of the findings for a general readership should be included on the title page. The statement should be written in the present tense and refer to the work in the third person. It should begin with "First author name(s) et al..." to match our preferred style.

10) Conflict of interest statement: JCB requires inclusion of a statement in the acknowledgements regarding competing financial interests. If no competing financial interests exist, please include the following statement: "The authors declare no competing financial interests." If competing interests are declared, please follow your statement of these competing interests with the following statement: "The authors declare no further competing financial interests."

11) A separate author contribution section is required following the Acknowledgments in all research manuscripts. All authors should be mentioned and designated by their first and middle initials and full surnames. We encourage use of the CRediT nomenclature (https://casrai.org/credit/).

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B. FINAL FILES:

Please upload the following materials to our online submission system. These items are required prior to acceptance. If you have any questions, contact JCB's Managing Editor, Lindsey Hollander (lhollander@rockefeller.edu).

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure and MP4 video files: See our detailed guidelines for preparing your production-ready images, https://jcb.rupress.org/fig-vid-guidelines.

-- Cover images: If you have any striking images related to this story, we would be happy to consider them for inclusion on the journal cover. Submitted images may also be chosen for highlighting on the journal table of contents or JCB homepage carousel. Images should be uploaded as TIFF or EPS files and must be at least 300 dpi resolution.

It is JCB policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.

The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements before choosing the appropriate license.

Additionally, JCB encourages authors to submit a short video summary of their work. These videos are intended to convey the main messages of the study to a non-specialist, scientific audience. Think of them as an extended version of your abstract, or a short poster presentation. We encourage first authors to present the results to increase their visibility. The videos will be shared on social media to promote your work. For more detailed guidelines and tips on preparing your video, please visit https://rupress.org/jcb/pages/submission-guidelines#videoSummaries.

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days. If complications arising from measures taken to prevent the spread of COVID-19 will prevent you from meeting this deadline (e.g. if you cannot retrieve necessary files from your laboratory, etc.), please let us know and we can work with you to determine a suitable revision period.

Please contact the journal office with any questions, cellbio@rockefeller.edu or call (212) 327-8588.

Thank you for this interesting contribution, we look forward to publishing your paper in Journal of Cell Biology.

Sincerely,

William Bement, PhD
Monitoring Editor
Journal of Cell Biology

Dan Simon, PhD
Scientific Editor
Journal of Cell Biology