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Downregulation of basal myosin-II is required for cell shape changes and tissue invagination

Daniel Krueger, Pietro Tardivo, Congtin Nguyen, and Stefano De Renzis

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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 24th Jul 2018

Thank you for submitting your manuscript entitled 'Downregulation of basal myosin activity is required for cell shape changes and tissue invagination' to The EMBO Journal. We have now received two referee reports, which are included below.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of the reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

REFEREE REPORTS:

Referee #1:

This is an excellent manuscript. The authors use optogenetic activation of contractility to show that basal relaxation is essential to formation of epithelial folds in the fly embryo. This is a model system of major importance in morphogenesis so their work is highly relevant for the field. The tools used are very well described and the quality of the data is really impressive. To date, all of the attention in ventral furrow formation has been on the apical surface, so these findings place new emphasis on events occurring basally. Overall, an outstanding effort worthy of publication in EMBO J. I recommend publication without delay.

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Referee #2:

The paper by Krueger et al. examines the role of a reduction in basal myosin during the process of gastrulation in the Drosophila embryo. While extensive work was published on the organization and regulation of apical myosin, the untested assumption was that basal myosin should be decreased to allow normal gastrulation. The current paper utilizes state-of-the-art optogenetic approaches to increase the level of basal myosin at two different phases, and test this assumption. In the first phase they carry out targeted basal recruitment of RhoGEF2, and observe a non-autonomous effect on apical myosin constrictions. While the constrictions themselves continue to take place, they fail to be stabilized leading to loss of the ratcheting mechanism. The assumption is that the increased basal resistance to cell stretching affects the apical processes. In the second phase, local optogenetic activation was elegantly achieved by using a membrane protein that is basally located, rather than by local illumination. In this stage the excess basal myosin inhibited cell shortening and folding of the tissue to a tube-like structure.
The work is elegant and timely, and would be of interest to a wide audience. Publication is recommended after taking the minor comments below into consideration.
1. Establishing that the system works (Fig. 1): Not fully apparent what is being measured and why these measurements are important. In particular, panels 1J (z-spreading) and 1L (cell density). Authors should use simple, clear language in presenting these experiments.
2. Consequences of stabilizing basal myosin from the beginning of gastrulation (Figs 3 and 4): The manuscript could benefit from presenting this portion of the study in a somewhat different fashion. Rather then have the resulting "disorganized cell behavior" leading the way towards further analysis, move up the observation (Fig 4A) that the large majority of cells do not constrict their apices. Apical constriction is the cell-shape change most readily associated with ventral furrow formation, and the unexpected influence of stabilizing basal myosin on this process (along with the demonstration that this results from non-productive "ratcheting"), is perhaps the most novel observation stemming from the study.
3. Construction of a basally-targeted optogenetic probe (Fig 5): the authors construct a series of such probes, but although all localize basally, only one of them is "functional" (Fig 5E). No data accompanies the "functionality" observation. Do the other probes fail due to a lack of myosin recruitment? While expanding this section (perhaps as supplementary data) is not critical for the observations made and the conclusions drawn from using the active (CIBN-PatJ-CAAX) construct, it raises technical issues which will be of interest to future research using similar techniques.
4. It would be worthwhile to present data quantifying the degree of myosin recruitment following expression of CIBN-PatJ-CAAX in dorsal epithelium cells, similar to what was shown for the "generic" CIBN, to demonstrate a ~two-fold stabilization.

1st Revision - authors' response 6th Sep 2018

Referee #1:

This is an excellent manuscript. The authors use optogenetic activation of contractility to show that basal relaxation is essential to formation of epithelial folds in the fly embryo. This is a model system of major importance in morphogenesis so their work is highly relevant for the field. The tools used are very well described and the quality of the data is really impressive. To date, all of the attention in ventral furrow formation has been on the apical surface, so these findings place new emphasis on events occurring basally. Overall, an outstanding effort worthy of publication in EMBO J. I recommend publication without delay.

We thank this reviewer for the positive comments on our manuscript.

Referee #2:

The paper by Krueger et al. examines the role of a reduction in basal myosin during the process of gastrulation in the Drosophila embryo. While extensive work was published on the organization and regulation of apical myosin, the untested assumption was that basal myosin should be decreased to allow normal gastrulation. The current paper utilizes state-of-the-art optogenetic approaches to increase the level of basal myosin at two different phases, and test this assumption. In the first phase they carry out targeted basal recruitment of RhoGEF2, and observe a non-autonomous effect on apical myosin constrictions. While the constrictions themselves continue to take place, they fail to be stabilized leading to loss of the ratcheting mechanism. The assumption is that the increased basal resistance to cell stretching affects the apical processes. In the second phase, local optogenetic activation was elegantly achieved by using a membrane protein that is basally located, rather than by local illumination. In this stage the excess basal myosin inhibited cell shortening and folding of the tissue to a tube-like structure.
The work is elegant and timely, and would be of interest to a wide audience. Publication is recommended after taking the minor comments below into consideration.

We thank this reviewer for the positive evaluation of our manuscript and useful suggestions

1. Establishing that the system works (Fig. 1): Not fully apparent what is being measured and why these measurements are important. In particular, panels 1J (z-spreading) and 1L (cell density). Authors should use simple, clear language in presenting these experiments.
In panel 1J z-spreading refers to the extent to which myosin-II diffuses away from the basal plane of photo-activation along the apico-basal axis. Because our aim was to achieve selective basal accumulation of myosin-II, this value represents an important indicator of the spatial precision at which we could control myosin-II levels. We have clarified the meaning of this measurement in the text (p. 8 lanes 8-12) and corresponding figure legend.
“During that time, myosin-II remained tightly localized at the basal surface of the cells, with a maximum spreading along the apico-basal axis (z-spreading) from the most basal plane of ~4 microns (Fig 1G-H and Movie EV3). This value was only

slightly higher than non-activated control cells (Fig 1J), demonstrating the efficacy of this protocol in the selective up-regulate of myosin-II at the base.
In panel 1L we replaced “cell density” with “ring density”. This value measures the density of actomyosin rings at the basal surface of the cells. We explained the meaning of this measurement in the corresponding figure legend and in the text (p. 8 last 7 lanes) with the following paragraph: “To exclude the possibility that the increased actomyosin ring constriction caused a loss of basal tissue integrity, we compared the density of actomyosin rings inside and outside the photoactivation area. The result of this measurement revealed a ~1.3-fold increase in ring density upon photo-activation (Fig 1L and Movie EV2), arguing that cells were still interconnected at the base. Thus, we conclude that the established conditions allow for the quantitative control of myosin-II levels on the time-scale of ventral furrow formation”

2. Consequences of stabilizing basal myosin from the beginning of gastrulation (Figs 3 and 4): The manuscript could benefit from presenting this portion of the study in a somewhat different fashion. Rather then have the resulting "disorganized cell behavior" leading the way towards further analysis, move up the observation (Fig 4A) that the large majority of cells do not constrict their apices. Apical constriction is the cell-shape change most readily associated with ventral furrow formation, and the unexpected influence of stabilizing basal myosin on this process (along with the demonstration that this results from non-productive "ratcheting"), is perhaps the most novel observation stemming from the study.

While we agree that the inhibitory effects on apical constriction represents an important finding of our study, we would prefer to maintain the structure of our manuscript as it is. We have considered the possibility of changing the order in which Figs 3 and 4 are presented. However, we think that this change alters the flow of the manuscript with respect to the introduction and motivation of our study -which was to analyse the effects of basal myosin-II stabilization on cell shape changes in general and not specifically on apical constriction. We did not anticipate an inhibitory effect on ratchet constrictions but we came to this conclusion only after the quantitative analysis on cell shape presented in Fig.3. To highlight the inhibitory effect on apical constriction, we have included this finding both in the synopsis image and in the highlights of our manuscript.

3. Construction of a basally-targeted optogenetic probe (Fig 5): the authors construct a series of such probes, but although all localize basally, only one of them is "functional" (Fig 5E). No data accompanies the "functionality" observation. Do the other probes fail due to a lack of myosin recruitment? While expanding this section (perhaps as supplementary data) is not critical for the observations made and the conclusions drawn from using the active (CIBN-PatJ-CAAX) construct, it raises technical issues which will be of interest to future research using similar techniques.

We have added an entirely new Figure (Expanded Fig. 4) showing that -even upon one photon illumination, which provides a more powerful mean of photo-activation than two-photon- only the CIBN-PatJ-CAAX basal anchor induces myosin-II

recruitment and actomyosin ring constriction. The following paragraph has been added to the Result (p. 13, lanes 7-14) “In contrast, even upon one photon illumination during early cellularization stages, when the basal surface of the forming cells is only a few microns distant from the objective and thus more sensitive to photo-activation, the other basal anchors did not support myosin-II plasma membrane recruitment (Fig EV5A-L) nor actomyosin ring constriction during mid-cellularization (Fig EV5M-X). Thus, lack of myosin-II recruitment provides a mechanistic explanation of why only in the presence of CIBN-PatJ-GFP-CAAX could contractility be induced.

4. It would be worthwhile to present data quantifying the degree of myosin recruitment following expression of CIBN-PatJ-CAAX in dorsal epithelium cells, similar to what was shown for the "generic" CIBN, to demonstrate a ~two-fold stabilization.

To address this point, we have quantified myosin-II in ventral cells which demonstrates a two-fold increase, comparable to what achieved with the “generic” CIBN anchor both in dorsal and ventral cells. This data is presented in the new Expanded Fig. 4. and discussed in the Result (p. 13, lanes 3-7). “Consistent with this hypothesis, while CIBN-PatJ without a CAAX box did not support contractility, CIBN-PatJ plus the addition of a CAAX box, which upon prenylation is directly inserted into the lipid bilayer (Powers, 1991), resulted in a two-fold increase of myosin-II levels at the basal surface (Fig EV4) and increase in contractility (Fig 6A-C).”

2nd Editorial Decision 2nd Oct 2018

Thank you for submitting a revised version of your manuscript. It has now been seen by one of the original referees whose comments are shown below.

As you will see he/she finds that all criticisms have been sufficiently addressed and recommend the manuscript for publication. However, before I can officially accept the manuscript there are a few editorial issues concerning text and figures that I need you to address.

REFEREE REPORT:

Referee #2:

The authors have addressed our comments, and made compelling arguments in places where they preferred to stick to their original version. The paper is now ready for publication.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Stefano De Renzis
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2018-100170

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

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B- Statistics and general methods

Please fill out these boxes (Do not worry if you cannot see all your text once you press return)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	By Cohen's d method
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	The sample size of biological replicates was N>= 3. For all analyses done the exact number of animal replicates is indicated by N. For cellular analyses the number of cells is indicated by n.
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	In this study we use an optogenetic method within a defined region of the Drosophila embryonic tissue. This enables us do perturb one part of the embryo and leave another part unperturbed, which serves as an internal control. Embryos were excluded from the analysis if they showed severe defects in the unperturbed part of the tissue, if they were not correctly positioned at the microscope or if a technical problem was encountered. These criterions were preestablished.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Experiments were performed in different genetic backgrounds, with different marker proteins. Regarding the optogenetic methods used, dark state (non-photoactivated) control experiments were performed. Animals were selected randomly based only based on the developmental stage. For cellular analyses, cells were choosen randomly and only excluded if the segmentation was defective.
For animal studies, include a statement about randomization even if no randomization was used.	Embryos were randomly selected only based on the developmental stage.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	The experimental precedure was standardized and the different data sets acquired by three independent experimentalists.
4.b. For animal studies, include a statement about blinding even if no blinding was done	At the initial stages of this study, the experiment was performed by one experimentalist and analyzed by a different person without knowing the site of the perturbation to assess the impact of the perturbation.
5. For every figure, are statistical tests justified as appropriate?	Yes.

Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The data were analyzed in histograms and resembled bell-shaped normal distribution.
Is there an estimate of variation within each group of data?	Yes. The data are represented either with the corresponding standard deviation or by boxplots with the sample number indicated.
Is the variance similar between the groups that are being statistically compared?	The variance between groups is similar in most experiments and when we observed notalby different variances between groups, we indicated it in the results and legends . The effect size was estimated using Cohen's d method.

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	N/A
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	N/A

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D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Please see the Method chapter 'Fly Strains and Genetics'
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	N/A
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	We confirm compliance.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
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17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	N/A
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	N/A
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	No.