The RhoGEF protein Plekhg5 regulates medioapical and junctional actomyosin dynamics of apical constriction during Xenopus gastrulation

Austin Baldwin, Ivan Popov, Ray Keller, John Wallingford, and Chenbei Chang

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(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.)

RE: Manuscript #E22-09-0411
TITLE: The RhoGEF protein Plekhg5 regulates medioapical actomyosin dynamics of apical constriction during Xenopus gastrulation

Dear Dr. Baldwin:

While this manuscript reports novel and interesting findings that build on an earlier publication by these authors on the role of Plekhg5 in apical constriction, a number of significant concerns were raised that would have to be addressed in order for this paper to be considered for publication in MBoC. Please respond to all of the reviewers' comments, with particular emphasis on the items listed below:

1. Address the issues raised by both reviewers regarding the evidence that the actin-enriched medial structures are indeed microvilli - Could these structures be an imaging artifact, actin comets, or a pool of medial actin associated with myosin? What accounts for the variability in the intensity of medial utrophin?
2. Provide the missing quantitation, including the number of embryos imaged
3. In light of the data revealing a disruption of junctional myosin in Plekhg5 morphants, please provide a rationale for why the model of medial disruption is favored.
4. Tone down the conclusion statements regarding 1) the forces that shape fusiform and round bottle cells 2) the role of medial actomyosin contractility in apical constriction

Sincerely,

Rachel Brewster
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Baldwin,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript is not acceptable for publication at this time, but may be deemed acceptable after specific revisions are made, as described in the Monitoring Editor's decision letter above and the reviewer comments below.

A reminder: Please do not contact the Monitoring Editor directly regarding your manuscript. If you have any questions regarding the review process or the decision, please contact the MBoC Editorial Office (mboc@ascb.org).

When submitting your revision include a rebuttal letter that details, point-by-point, how the Monitoring Editor's and reviewers' comments have been addressed. (The file type for this letter must be "rebuttal letter"; do not include your response to the Monitoring Editor and reviewers in a "cover letter.") Please bear in mind that your rebuttal letter will be published with your paper if it is accepted, unless you haveopted out of publishing the review history.

Authors are allowed 180 days to submit a revision. If this time period is inadequate, please contact us at mboc@ascb.org.

Revised manuscripts are assigned to the original Monitoring Editor whenever possible. However, special circumstances may preclude this. Also, revised manuscripts are often sent out for re-review, usually to the original reviewers when possible. The Monitoring Editor may solicit additional reviews if it is deemed necessary to render a completely informed decision.

In preparing your revised manuscript, please follow the instruction in the Information for Authors (www.molbiolcell.org/info-for-authors). In particular, to prepare for the possible acceptance of your revised manuscript, submit final, publication-quality figures with your revision as described.

To submit the rebuttal letter, revised manuscript, and figures, use this link: Link Not Available

Please contact us with any questions at mboc@ascb.org.

Thank you for submitting your manuscript to Molecular Biology of the Cell. We look forward to receiving your revised paper.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org

Reviewer #1 (Remarks to the Author):

This manuscript reports on normal changes in cell shape and actomyosin dynamics during Xenopus bottle cell formation, and it addresses how the RhoGEF Plekhg5 contributes to changes in cell shape. The authors report that both rounded and elongated cells are found during bottle cell formation, and they use a utrophin-GFP construct to examine F-actin distribution and dynamics in cells of both shapes. Shrinking of cell surfaces correlated better with F-actin localization medially than junctionally, leading the authors to conclude that medial actomyosin contractility drives apical constriction. Injecting an antisense morpholino against Plekhg5 led to the expected failure of apical constriction and disrupted F-actin localization that had been seen before, but here the authors can follow the result with live imaging. They report that F-actin and myosin distributions are disrupted more medially than junctionally.

Plekhg5 is a tremendously interesting protein, in my view, because it is one of only a few proteins in any system that both localizes specifically to apically constricting cells and that has been implicated in apical constriction. For this reason, I was excited to read the 2018 Popov et al paper. And I applaud the authors' efforts in the current manuscript to get a better description of normal cell shape changes and cytoskeletal dynamics, and to better understand Plekhg5's roles. But I have a number of major concerns with the manuscript.

Major concerns
- In the first section of the results, the coexistence of rounded and fusiform cell shapes is interpreted as implying that "bottle cells are formed by both isotropic and anisotropic apical constriction". It is not possible to understand forces based on cell shapes alone. For example, the fusiform cells could result from anisotropic apical constriction or they could be stretched passively by neighboring cells undergoing apical constriction.
- F-actin distribution is reported in "bundles" that look to me like they could be only dots stretched in the Z axis as expected in a typical point spread function, and in "microvilli" that could potentially be actin comets instead.
- In Figures 2 and 3, both junctional actin results appear bimodal, with a strongly correlating population and an anti-correlating population. I'm not sure how to interpret the resulting difference in correlations between medial and junctional actin given the biomodal distribution. More generally, I'm not convinced that correlations can be intepreted as causation, as for example in the conclusion "These results indicate that medial actomyosin contractility drives apical constriction".
- In the last section of the Results, both medial and junctional MyoIIB look strongly affected by Plekhg5 MO (Fig 6 C,D), as they did for actin (Fig 4C,D) but the authors conclude that "the major disruption was seen in the medial region".

Minor comments
- In Figure 1, it would be useful to see cells represented in 3D renderings.
- Scale bars are missing from some figures.
- Quantification is needed for several statements in the paper for a reader to know if a result stated is generally true.
- It would be useful to validate the utrophin-GFP with phalloidin. Do all structures appear using both, or do some appear with only one?
- Conclusions that were already clear from the 2018 paper on Plekhg5 seem like they should not be announced as a major conclusion here in a section heading, "Knockdown of plekhg5 leads to failure in medioapical accumulation of F-actin and apical constriction". I agree with the authors' goal of looking at live dynamics, but the headings should presumably capture only any new conclusions.

Reviewer #2 (Remarks to the Author):

Advance Summary and Potential Significance to Field

Apical constriction is a major cell shape change in animal development, and therefore, understanding the molecular mechanisms of apical constriction is an important question in the cell and developmental biology field. Here, Baldwin et al. investigate the behavior of F-actin during apical constriction during Xenopus gastrulation. Building on previous studies of Plekhg5 in apical constriction, they further reveal novel roles for the protein in regulating medioapical myosin during this process. Their imaging is beautiful, and the quantification was done carefully. This work will be of broad interest to the cell and developmental biology community. I have several relatively minor suggestions that need clarification and could improve the manuscript.

Major points:
1. In Fig. 1C, although the microvilli structures on the left panel are clear, they're not clear in the panel on the right side. You can

definitely see enhanced utrophin-GFP signals, but are they really "densely packed microvilli" (page 5, lines 29-30)? I think it could be an additional pool of cortical F-actin that forms a complex with myosin.

2. Utrophin-GFP signals seem stronger in some cells compared to their neighbors. It could be partly due to the relatively small apical areas in those cells. But some cells show very strong utrophin-GFP signals despite their relatively similar apical area to neighboring cells (e.g., one in the middle of the last panel of Fig. 2A, 6324 sec; also shown in some cells in Fig. 3A). Why are there such differences in the level of F-actin in some cells?

3. Although microvilli structures are shown in plekhg5 MO embryos in Fig. 4A, it looks like they're fewer compared to control (Fig. 1C). Is this the case? Please clarify.

4. Sample numbers.
Only the number of cells analyzed were provided in figure legends in Figs. 2 and 3. Please provide the number of embryos analyzed as well. Sample numbers are missing in the quantification of Fig. 4B-D and Fig. 6C,D.

Minor points:
1. p.3, second paragraph
The authors adequately cited several original papers that revealed the role of medioapical myosin in apical constriction in several systems. But several citations are review papers, and some original papers in several premier model systems that use medioapical myosin contraction for apical constriction are missing (mainly Drosophila tissues after gastrulation). It would be good to include references for the following systems.
- Drosophila dorsal closure: Blanchard et al., 2010
- Drosophila salivary gland: Booth et al., 2014, Chung et al., 2017
- Drosophila neuroblast delamination: An et al., 2017, Simoes et al., 2017 (Simoes et al., 2017 also showed a hybrid strategy of employing both junctional and medioapical myosin in Drosophila neuroblast ingression.)

2. Figure 1
- All panels need scale bars in Fig. 1.
- Fig. 1A. It is hard to distinguish cell shapes (round vs. fusiform) in this image. It would help to mark the cell boundaries of a representative cell in each region.
- Fig. 1C. It will be helpful to add arrows to indicate microvilli. Since the two images shown in Fig. 1C look like different magnifications, a higher mag image for the right-side panel will also help for a proper comparison.

3. Page 6, line 12
Add reference for Tissue Analyzer. It was cited only later in line 26 of the same page.

4. Page 8, line 11
"The enhanced actomyosin signals concentrated mainly under the apical cell membrane instead of at the cell junctions."
Does it mean that the enhanced signals were concentrated across the entire apical surface, which covers both the medial and junctional regions? Please clarify.

5. It would be great if figure labels were more friendly to readers, especially for Figures 5 and 6. In Figure 5, two different stages are shown without labels, and you have to go back and forth between the figure and the legends and the text to figure out their stages and the difference between them. Similarly, in Figure 6, labels for control and plekhg5-MO (shown at the bottom panels) can be moved to the top, so it's easier to see them.

6. Supplemental Figure 1 needs the figure legend.

RE: Manuscript #E22-09-0411
TITLE: The RhoGEF protein Plekhg5 regulates medioapical actomyosin dynamics of apical constriction during Xenopus gastrulation

We thank both reviewers and the monitoring editor for their constructive critiques. We have modified our manuscript and included additional data to address their concerns. In the following, we detail our revision, point-by-point, in response to the comments made by the reviewers and the monitoring editor.

Responses to Reviewer #1:

This manuscript reports on normal changes in cell shape and actomyosin dynamics during Xenopus bottle cell formation, and it addresses how the RhoGEF Plekhg5 contributes to changes in cell shape. The authors report that both rounded and elongated cells are found during bottle cell formation, and they use a utrophin-GFP construct to examine F-actin distribution and dynamics in cells of both shapes. Shrinking of cell surfaces correlated better with F-actin localization medially than junctionally, leading the authors to conclude that medial actomyosin contractility drives apical constriction. Injecting an antisense morpholino against Plekhg5 led to the expected failure of apical constriction and disrupted F-actin localization that had been seen before, but here the authors can follow the result with live imaging. They report that F-actin and myosin distributions are disrupted more medially than junctionally.

Plekhg5 is a tremendously interesting protein, in my view, because it is one of only a few proteins in any system that both localizes specifically to apically constricting cells and that has been implicated in apical constriction. For this reason, I was excited to read the 2018 Popov et al paper. And I applaud the authors' efforts in the current manuscript to get a better description of normal cell shape changes and cytoskeletal dynamics, and to better understand Plekhg5's roles. But I have a number of major concerns with the manuscript.

Major concerns
- In the first section of the results, the coexistence of rounded and fusiform cell shapes is interpreted as implying that "bottle cells are formed by both isotropic and anisotropic apical constriction". It is not possible to understand forces based on cell shapes alone. For example, the fusiform cells could result from anisotropic apical constriction or they could be stretched passively by neighboring cells undergoing apical constriction.

We agree with the reviewer and acknowledge that we cannot conclude the sources of forces that generate round and fusiform cell shapes. We have revised our conclusion in the paragraph.

- F-actin distribution is reported in "bundles" that look to me like they could be only dots stretched in the Z axis as expected in a typical point spread function, and in "microvilli" that could potentially be actin comets instead.

We acknowledge that point spread function (PSF) can create illustration of z-elongation and actin comets that can lead to misinterpretation of results. We have therefore performed additional experiments using an airyscan microscopy to examine actin organization in fixed embryos stained with

fluorescent dye-conjugated phalloidin. We included two regions, one in the area where bottle cells have formed and the other in the neighboring area where F-actin starts to accumulate but the surface areas of the cells have not reduced yet. The z-step of the experiments was kept at 0.2µm, the best for airyscan imaging. With 3D projection and oblique views, we observed abundant F-actin bundles perpendicular to the cell surface in bottle cells, whereas shorter and dispersed F-actin assembly were seen in neighboring cells that had just begun to accumulate F-actin in the apical cortex. Additionally, we have included additional quantification showing that the length of the bundles increases with apical constriction in Supplementary Figure 1. The results supported our original conclusion about the F-actin-rich microvilli in bottle cells. We have now added the data in the revised Figure 1.

- In Figures 2 and 3, both junctional actin results appear bimodal, with a strongly correlating population and an anti-correlating population. I'm not sure how to interpret the resulting difference in correlations between medial and junctional actin given the biomodal distribution. More generally, I'm not convinced that correlations can be intepreted as causation, as for example in the conclusion "These results indicate that medial actomyosin contractility drives apical constriction".

We thank the reviewer for this astute observation. We agree that the result complicates the comparison of the relative contributions of medial and junctional actin to apical constriction in individual cells, but across the population of bottle cells we still believe these data suggest a stronger role for medial F-actin. To clarify this, we have mentioned the anti-correlated population of cells in the text and annotated them in Figure 2D and Figure 3F. In addition, we have modified our interpretation of this data to state that the role of junctional F-actin in apical constriction is more variable, and that this subpopulation of cells appears more prevalent within the fusiform bottle cells.

- In the last section of the Results, both medial and junctional MyoIIB look strongly affected by Plekhg5 MO (Fig 6 C,D), as they did for actin (Fig 4C,D) but the authors conclude that "the major disruption was seen in the medial region".

We made the original conclusion based on the observation that increase in medial myoIIB signal was much stronger during the late stage of apical constriction of the bottle cells (Fig. 5B, C), which makes the effects of medial disruption of myoIIB signal in *plekhg5* knockdown cells more prominent. However, we agree with the reviewer that quantitative analysis did reveal both medial and junctional reduction of myoIIB signals in the knockdown cells. We have therefore modified the sentence to reflect this.

Minor comments
- In Figure 1, it would be useful to see cells represented in 3D renderings.

We have now included 3D rendition of F-actin in bottle cells in Figure 1. Since live imaging can produce more pronounced effect of actin comets that can obscure data interpretation, we decided to use phalloidin-stained samples to detect F-actin. The 3D renderings of images were made for these fixed and stained samples.

- Scale bars are missing from some figures.

We have now included scale bars to the first image of each panel in the figures.

- Quantification is needed for several statements in the paper for a reader to know if a result stated is generally true.

We have removed and/or revised several statements in the manuscript that indicated quantitation that we did not provide, i.e. “Though junctional F-actin was present in the bottle cells, most of the increased signals were detected in the medioapical region (left panel, **Fig. 1B**).”

- It would be useful to validate the utrophin-GFP with phalloidin. Do all structures appear using both, or do some appear with only one?

We have now performed the study on phalloidin-stained embryos and included the data in the revised Figure 1. Figure 1B shows the Utrophin-GFP samples whereas Figure 1C now shows the phalloidin-stained samples. In both cases, F-actin bundles perpendicular to the apical surface were observed in cells undergoing apical constriction whereas in un-constricting cells, F-actin bundles were shorter and located mainly at the cell junctions.

- Conclusions that were already clear from the 2018 paper on Plekhg5 seem like they should not be announced as a major conclusion here in a section heading, "Knockdown of plekhg5 leads to failure in medioapical accumulation of F-actin and apical constriction". I agree with the authors' goal of looking at live dynamics, but the headings should presumably capture only any new conclusions.

Our previous studies demonstrated reduction of apical F-actin in *plekhg5* knockdown cells, but we did not track medial versus junctional F-actin pools. We have now modified the subtitle to reflect our emphasis on the latter point.

Responses to Reviewer #2:

Advance Summary and Potential Significance to Field

Apical constriction is a major cell shape change in animal development, and therefore, understanding the molecular mechanisms of apical constriction is an important question in the cell and developmental biology field. Here, Baldwin et al. investigate the behavior of F-actin during apical constriction during Xenopus gastrulation. Building on previous studies of Plekhg5 in apical constriction, they further reveal novel roles for the protein in regulating medioapical myosin during this process. Their imaging is beautiful, and the quantification was done carefully. This work will be of broad interest to the cell and developmental biology community. I have several relatively minor suggestions that need clarification and could improve the manuscript.

Major points:
1. In Fig. 1C, although the microvilli structures on the left panel are clear, they're not clear in the panel on the right side. You can definitely see enhanced utrophin-GFP signals, but are they really "densely packed microvilli" (page 5, lines 29-30)? I think it could be an additional pool of cortical F-actin that forms a complex with myosin.

We agree with the reviewer that our original image did not show microvilli explicitly. As live imaging takes time and can exacerbate the effect of point spread function to potentially create illusion of z-axial elongation (as commented by reviewer #1), we now switched to phalloidin-stained embryos to show F-actin bundles perpendicular to apical cell surface. We include the new data in the revised Figure 1C.

2. Utrophin-GFP signals seem stronger in some cells compared to their neighbors. It could be partly due to the relatively small apical areas in those cells. But some cells show very strong utrophin-GFP signals despite their relatively similar apical area to neighboring cells (e.g., one in the middle of the last panel of Fig. 2A, 6324 sec; also shown in some cells in Fig. 3A). Why are there such differences in the level of F-actin in some cells?

We did observe certain degree of discordance of F-actin signal strength and cell surface area in some cells during the initial phases of apical constriction. This may be due to heterogeneity in original sizes of the cells as well as the timing when F-actin starts to accumulate. Strong negative correlation of F-actin signal and apical area is observed in later stages of apical constriction. As cell surface reduces and F-actin intensity increases, cells form clusters that make it harder to distinct individual cells. For example, in Fig. 2A, 6324 sec, the apparent cell with strong F-actin signal in the middle (close to the yellow arrow) actually consists of 5 constricted cells when tracked back to the beginning of the movie. Similarly, the apparent cell with strong F-actin signal in Fig. 3A (bottom yellow arrow) originated from a group of over 10 cells. We now clarify the point in the figure legends.

3. Although microvilli structures are shown in plekhg5 MO embryos in Fig. 4A, it looks like they're fewer compared to control (Fig. 1C). Is this the case? Please clarify.

We agree with the assessment of the reviewer. Since we did not examine microvilli directly, we toned down our conclusion and described formation of F-actin puncta instead. The number of puncta seemed to resemble those in control cells at the onset of F-actin accumulation, but the cells with *plekhg5* knockdown failed to increase the number of the puncta and overall F-actin intensity as development progresses.

4. Sample numbers.
Only the number of cells analyzed were provided in figure legends in Figs. 2 and 3. Please provide the number of embryos analyzed as well. Sample numbers are missing in the quantification of Fig. 4B-D and Fig. 6C,D.

We have now added the number of the embryos in the legends of Figs. 2 and 3 as well as the sample numbers in the legends of Fig. 4 and 6.

Minor points:
1. p.3, second paragraph
The authors adequately cited several original papers that revealed the role of medioapical myosin in apical constriction in several systems. But several citations are review papers, and some original papers in several premier model systems that use medioapical myosin contraction for apical constriction are missing (mainly Drosophila tissues after gastrulation). It would be good to include references for the following systems.
- Drosophila dorsal closure: Blanchard et al., 2010
- Drosophila salivary gland: Booth et al., 2014, Chung et al., 2017
- Drosophila neuroblast delamination: An et al., 2017, Simoes et al., 2017 (Simoes et al., 2017 also

showed a hybrid strategy of employing both junctional and medioapical myosin in Drosophila neuroblast ingression.)

We thank the reviewer for pointing out several important works regarding medioapical actomyosin contraction in apical constriction in distinct developmental processes. We have now added the references in our revised manuscript.

2. Figure 1
- All panels need scale bars in Fig. 1.
- Fig. 1A. It is hard to distinguish cell shapes (round vs. fusiform) in this image. It would help to mark the cell boundaries of a representative cell in each region.
- Fig. 1C. It will be helpful to add arrows to indicate microvilli. Since the two images shown in Fig. 1C look like different magnifications, a higher mag image for the right-side panel will also help for a proper comparison.

We have now added scale bars for the panels in Fig. 1, marked the boundaries of several representative round and fusiform cells, and added 3D projected images of phalloidin-stained samples showing perpendicular F-actin bundles.

3. Page 6, line 12
Add reference for Tissue Analyzer. It was cited only later in line 26 of the same page.

We have now added the references for Tissue Analyzer in this section.

4. Page 8, line 11
"The enhanced actomyosin signals concentrated mainly under the apical cell membrane instead of at the cell junctions."
Does it mean that the enhanced signals were concentrated across the entire apical surface, which covers both the medial and junctional regions? Please clarify.

We appreciate the reviewer's noting of this point. We don't provide direct quantification for this statement, which also aligns with one of Reviewer 1's general comments. We have removed this statement and further clarified how we performed our image quantification in this section of the main text.

5. It would be great if figure labels were more friendly to readers, especially for Figures 5 and 6. In Figure 5, two different stages are shown without labels, and you have to go back and forth between the figure and the legends and the text to figure out their stages and the difference between them. Similarly, in Figure 6, labels for control and plekhg5-MO (shown at the bottom panels) can be moved to the top, so it's easier to see them.

We have now modified the labels for these figures so that they can be read more readily.

6. Supplemental Figure 1 needs the figure legend.

We have now added figure legend to Supplemental Figure 1.

Responses to Monitoring editor:

While this manuscript reports novel and interesting findings that build on an earlier publication by these authors on the role of Plekhg5 in apical constriction, a number of significant concerns were raised that would have to be addressed in order for this paper to be considered for publication in MBoC. Please respond to all of the reviewers' comments, with particular emphasis on the items listed below:

1. Address the issues raised by both reviewers regarding the evidence that the actin-enriched medial structures are indeed microvilli - Could these structures be an imaging artifact, actin comets, or a pool of medial actin associated with myosin? What accounts for the variability in the intensity of medial utrophin?

We have now included 3D projected views of phalloidin-stained samples zooming in on apical F-actin and showed that F-actin bundles perpendicular to apical surface were enriched in bottle cells. These F-actin bundles were longer than those found at the cell junctions of non-constricting cells, implying that the signals were unlikely due to imaging artifact. We did observe some variation in F-actin intensity and apical surface areas. We contributed the effect to the heterogeneity of the initial sizes of the cells, the onset of F-actin accumulation even among neighboring cells, and the congregation of constricted cells in clusters that appeared to be one cell. We have clarified these points in the figure legends in the revised manuscript.

2. Provide the missing quantitation, including the number of embryos imaged

We have added the number of embryos analyzed in the figure legends.

3. In light of the data revealing a disruption of junctional myosin in Plekhg5 morphants, please provide a rationale for why the model of medial disruption is favored.

We observed sustained increase in medial myosin intensity during apical constriction whereas junctional myosin signals did not keep pace at late stages. This seemed to imply a more critical role of medial myosin assembly in apical constriction. However, it is likely that medial and junctional myosin are both important during early stages of apical constriction. Since *plekhg5* knockdown cells failed to accumulate medial myosin at early stages and both medial and junctional myosin signals were reduced with time, it is possible that interactions of medial and junctional myosin reinforce the stability of the network. We have now modified our manuscript to tone down our conclusions about the role of medial myosin.

4. Tone down the conclusion statements regarding 1) the forces that shape fusiform and round bottle cells 2) the role of medial actomyosin contractility in apical constriction

We have modified the text to tone down our conclusions about the forces and the medioapical actomyosin contractility in apical constriction.

RE: Manuscript #E22-09-0411R
TITLE: "The RhoGEF protein Plekhg5 regulates medioapical and junctional actomyosin dynamics of apical constriction during Xenopus gastrulation"

Dear Dr. Baldwin:

Thank you for submitting your article entitled "The RhoGEF protein Plekhg5 regulates medioapical actomyosin dynamics of apical constriction during Xenopus gastrulation" for consideration by Molecular Biology of the Cell. Based on the edits you made in response to Reviewers 1 and 2, I am now pleased to accept your manuscript for publication.

Sincerely,
Rachel Brewster
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Baldwin:

Congratulations on the acceptance of your manuscript! Thank you for choosing to publish your work in Molecular Biology of the Cell (MBoC).

Within 10 days, an unedited PDF of your manuscript will be published on MBoC in Press, an early release version of the journal. The date your manuscript appears on this site is the official publication date.

Your copyedited and typeset manuscript will be scheduled for publication in the next available issue of MBoC. Our production team will notify you when the page proofs of your paper are ready for your review.

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We look forward to publishing your paper in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
MBoC Editorial Office
mbc@ascb.org
