PCH-2/TRIP13 regulates spindle checkpoint strength

Lenaig Defachelles, Anna Russo, Christian Nelson, and Needhi Bhalla

Corresponding author(s): Needhi Bhalla, University of California, Santa Cruz

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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.)

RE: Manuscript #E20-05-0310
TITLE: "PCH-2/TRIP13 regulates spindle checkpoint strength"

Dear Dr. Bhalla:

After receiving both reviews, they have provided insightful and helpful suggestions to not only allow improve the clarity of the writing but to highlight the significance of the scientific discoveries. Both reviewers commented on the use of C. elegans as strength given the ability to measure the contributions of unattached kinetochores, cell volume and cell fate in the regulation of spindle strength. The genetic and live-cell imaging approaches were considered "clever" and address the important question about how the spindle checkpoint is regulated by cell size and differentiation.

The reviewers have both have made excellent suggestions to make the work accessible for a broad scientific audience. I encourage you to address these as you revise your manuscript. Both reviewers agreed a revision to the manuscript is necessary before publication in MBoC, which I also fully support. There was a concern that the writing of the manuscript for a broad audience given the array of names and systems discussed throughout should be addressed. One suggestion would be to have a TABLE to talk about the roles of checkpoint proteins in different systems, so the reader does not have to "guess what the importance of those differences are as the experiments", results and outcomes are discussed. One way to do this is to have C. elegans/Human protein name used throughout as a suggestion, but I am open to discuss the best way to do this while revisions are taking place. Next, there was a concern that the genetic and drug studies appeared to be treated similarly without discussing obvious complications of the interpretations of these experiments are given how they are performed. I suggest you address this concern in the revised manuscript in interpretation and discussion of results.

I look forward to receiving a revised manuscript in the near future. Please don't hesitate to ask me for advice or if you have concerns on making your manuscript accessible to a broad audience.

I value that you submitted your exciting findings to MBoC, and happy that you considered my expertise to be of value in the peer review process as your Monitoring Editor. I look forward to helping you publish your work in a timely fashion in MBoC. Don't hesitate to contact me for any reason.

Sincerely,
Dr. Ahna Skop
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Bhalla,

The review of your manuscript, referenced above, is now complete. The Monitoring Editor has decided that your manuscript requires minor revisions before it can be published in Molecular Biology of the Cell, as described in the Monitoring Editor's decision letter above and the reviewer comments (if any) 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 have opted out of publishing the review history.

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

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 version, and figures, please use this link (please enable cookies, or cut and paste URL): Link Not Available

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Thank you for submitting your manuscript to Molecular Biology of the Cell. Please do not hesitate to contact this office if you have any questions.

Sincerely,

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

Reviewer #1 (Remarks to the Author):

Comments to Authors
In this manuscript Defachelles et al., explore the role of PCH-2 in the regulation of the strength of the Spindle Checkpoint. They use a model system that allows them to measure the contributions of unattached kinetochores, cell volume and cell fate in the regulation of spindle strength as it regards to PCH-2. Several lines of evidence support a role for PCH-2 in silencing the Spindle Checkpoint by modulating the state of Mad2. However, there is also data to support a role of PCH-2 in the establishment of the Spindle Checkpoint. Through a series of elegant experiments the authors come up with a model that predicts that the role of PCH-2 in establishing the Spindle Checkpoint strength is through regulating the availability of the Open form of Mad2 at or near unattached kinetochores. This role is important in large cells such as oocytes. When the authors decrease the size of the of embryo cells, PCH-2 is no longer required for the spindle checkpoint in cells with decreased volume.

Strengths
This manuscript has many strengths including the model system used to study the role of PCH-2/TRIP13 in maintaining checkpoint strength. The data from the experiments with AB and P1 cells are clear, convincing and use an elegant system to study the contribution of factors that set up the asymmetry of AB and P1 cells in the first division of C elegans as well as the role of CMT-1 in the PCH2-mediated stronger checkpoint delay observed in the P1 cells.

Weaknesses
The manuscript as written is confusing. Rightly so, the authors want the reader to know that the proteins being studied have been shown to have different roles in the Spindle Checkpoint in different cell types and model systems. This could be made much easier for the reader to follow if that information is presented in the form of a Table. Such a Table would list the findings for different orthologs and different cell systems with the references in the last column of the table.

Also, some of the manipulations in earlier experiments showed very modest effects, yet the authors made very strong conclusions based on small differences (One example are the data in figure 4A).

The use of nocodazole and genetic manipulations to trigger the checkpoint interchangeably was a bit distracting.

Reviewer #2 (Remarks to the Author):

The manuscript by Defachelles et al, the authors tested the role of PCH-2 in regulating spindle checkpoint strength in C. elegans early embryonic development. Previous work showed that PCH-2 was required for the spindle checkpoint in the AB cell of the 2-cell embryo. They manipulated cell volume with ani1-2 mutants and triggered a checkpoint response with zyg-1 mutation and showed that pch-2 muants had a checkpoint response and Mad2 chromosome localization in small embryos but not in wildtype sized embryos. These results suggest that Pch-2 is needed in large AB cells but not small AB cells. They then checked whether Pch-2 was needed in cells that got smaller during the normal process of cell division in the embryo with two methods to trigger a checkpoint response. They found that in the 16-cell embryo pch-2 mutants have a modest checkpoint delay, suggesting that PCH-2 is needed for a robust checkpoint delay in these smaller cells. In P1 cells, they show that PCH-2 is needed for the stronger checkpoint response in comparison to AB cells and that there is more PCH-2 surrounding the chromosomes. They

depleted par-1 to make the AB and P1 cells the same size and found that PCH-2 levels were similar between the two cells. Previous work showed that this manipulations leads to similar checkpoint strength between the two cells. The cmt-1 mutants also lost the stronger checkpoint signal in the P1 cells compared to the AB cells. The levels of PCH-2 around the chromosome was decreased in the cmt-1 mutant. These results suggest that CMT-1 enriches PCH-2 in the P1 cells.

Overall, this study uses clever genetic manipulations and live cell imaging to address an important question about how the spindle checkpoint is regulated based on cell size and cell differentiation. The results suggest key differences in regulation of spindle checkpoint activity during development, and highlights the importance of studying the checkpoint in a model organism. There are several important conclusions from this study: i) PCH-2 is not needed for a checkpoint response in smaller AB cells, suggesting that cell size affects whether PCH-2 is important for the checkpoint response; ii) PCH-2 is not used for checkpoint silencing in the C. elegans embryo; iii) the germline progenitor P1 cell uses PCH-2 for a more robust checkpoint response, which depends on CMT-1 bringing more PCH-2 to the surrounding chromosome area; iv) the checkpoint response in P1 cells is regulated both by cell size and some factors of cell fate.

However, there are some additional experiments/ clarifications that are needed:
1) For Figure S1A, a wildtype strain with Zyg1 RNAi is needed to show that the ani1-2 RNAi did not disrupt cell cycle timing. By the way the description of the conclusion is explained in the text, I was expecting to see this data in the figure (lines 152-153).
2) More explanation is needed for the model. It is not clear why cmt-1 mutants would have more O-Mad2 available if CMT-1 is needed to convert C-Mad2 into O-Mad2. This would suggest that more C-Mad2 would be available.
3) In the videos, it would be helpful to have a more detailed description to point out what we are supposed to take notice of. It is not clear when the onset of onset of cortical contractility occurs, so an arrow as to when this occurs would be helpful. Also, defining OCC in the figure legends would also be helpful.
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• In the abstract, the authors describe PCH-2 as remodeling the checkpoint effector Mad2 from an active conformation to an inactive one, but then the next sentences describe genetic data suggesting that PCH-2 is needed for spindle checkpoint activation. I think it is a hard transition to reconcile. Perhaps it would help to add a sentence before the data about how you and others show that PCH-2 is needed for spindle checkpoint activation.
• In the lines 190-195, the authors make two conclusions for their data in Figure 1. In the first conclusion, they state the results that pch-2 mutant AB cells have a robust spindle checkpoint as cells decrease in size, and their conclusion is that PCH-2 does not seem to affect spindle checkpoint silencing in C. elegans. While I agree with this conclusion, it did not seem to fit with the result just mentioned. The result mentioned suggest that PCH-2 has an important role in checkpoint activation in larger AB cells but not smaller AB cells. I think a third conclusion should be added in which the supporting data mentioned for lack of checkpoint silencing is that the pch-2 mutants have the same duration of spindle checkpoint activation as the control in the small embryos.
• In lines 523-526, the authors state that PCH-2's characterized biochemical activity regulates the availability of O-MAD2. Can the authors be more specific about what the characterized biochemical activity is doing. Do you mean disassembling C-Mad2 from CMT-1 or is there something else? If this is the case, it is unclear why disassembling a checkpoint active C-MAD2 would allow greater checkpoint strength.

Needhi Bhalla, Ph.D.
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July 10, 2020

Dear Ahna-

Thank you for the careful review and consideration of our manuscript, “PCH-2/TRIP13 regulates spindle checkpoint strength.” We appreciated the reviewers’ comments on our manuscript and their thoughtful and helpful suggestions to make this a stronger, more accessible and compelling story. Here, we present a point-by point response to their comments. **Our responses are in bold.**

Please contact me if you have any questions.

Regards,

Needhi Bhalla

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Thank you!

Weaknesses
The manuscript as written is confusing. Rightly so, the authors want the reader to know that the proteins being studied have been shown to have different roles in the Spindle Checkpoint in different cell types and model systems. This could be made much easier for the reader to follow if that information is presented in the form of a Table. Such a Table would list the findings for different orthologs and different cell systems with the references in the last column of the table.

We have included a table (Table 1) and referenced it in sections of the Introduction and Discussion that describe the findings about PCH-2/TRIP13 and CMT-1/p31^{comet} in *C. elegans* and human cell culture.

Also, some of the manipulations in earlier experiments showed very modest effects, yet the authors made very strong conclusions based on small differences (One example are the data in figure 4A).

We have moderated our language in describing the results from Figure 4A:

"However, cells in the AB lineage in 16-cell *pch-2* mutant embryos treated with nocodazole exhibited a slight but significant cell cycle delay when compared to similar cells in *pch-2* mutants treated with DMSO and *san-1* mutants treated with nocodazole. Thus, as cells of the AB lineage naturally decrease in cell size to 16 cell embryos, *pch-2* mutants treated with nocodazole exhibit some delay of the cell cycle, albeit not as prolonged as control embryos, consistent with a defect in spindle checkpoint strength."

The use of nocodazole and genetic manipulations to trigger the checkpoint interchangeably was a bit distracting.

We included this sentence at the start of this section: "We initially performed these experiments in embryos treated with nocodazole, which depolymerizes microtubules and induces spindle checkpoint activation in a manner similar to when cells have monopolar spindles (Galli and Morgan, 2016; Gerhold *et al.*, 2018)."

Reviewer #2 (Remarks to the Author):

The manuscript by Defachelles et al, the authors tested the role of PCH-2 in regulating spindle checkpoint strength in C. elegans early embryonic development. Previous work showed that PCH-2 was required for the spindle checkpoint in the AB cell of the 2-cell embryo. They manipulated cell volume with ani1-2 mutants and triggered a checkpoint response with zyg-1 mutation and showed that pch-2 muants had a checkpoint response and Mad2 chromosome localization in small embryos but not in wildtype sized embryos. These results suggest that Pch-2 is needed in large AB cells but not small AB cells. They then checked whether Pch-2 was needed in cells that got smaller during the normal process of cell division in the embryo with two methods to trigger a checkpoint response. They found that in the 16-cell embryo pch-2 mutants have a modest checkpoint delay, suggesting that PCH-2 is needed for a robust checkpoint delay in these smaller cells. In P1 cells, they show that PCH-2 is needed for the stronger checkpoint response in comparison to AB cells and that there is more PCH-2 surrounding the chromosomes. They depleted par-1 to make the AB and P1 cells the same size and found that PCH-2 levels were similar between the two cells. Previous work showed that this manipulations leads to similar checkpoint strength between the two cells. The cmt-1 mutants also lost the stronger checkpoint signal in the P1 cells compared to the AB cells. The levels of PCH-2 around the chromosome was decreased in the cmt-1 mutant. These results suggest that CMT-1 enriches PCH-2 in the P1 cells.

Overall, this study uses clever genetic manipulations and live cell imaging to address an important question about how the spindle checkpoint is regulated based on cell size and cell differentiation. The results suggest

key differences in regulation of spindle checkpoint activity during development, and highlights the importance of studying the checkpoint in a model organism. There are several important conclusions from this study: i) PCH-2 is not needed for a checkpoint response in smaller AB cells, suggesting that cell size affects whether PCH-2 is important for the checkpoint response; ii) PCH-2 is not used for checkpoint silencing in the C. elegans embryo; iii) the germline progenitor P1 cell uses PCH-2 for a more robust checkpoint response, which depends on CMT-1 bringing more PCH-2 to the surrounding chromosome area; iv) the checkpoint response in P1 cells is regulated both by cell size and some factors of cell fate.

However, there are some additional experiments/ clarifications that are needed:
1) For Figure S1A, a wildtype strain with Zyg1 RNAi is needed to show that the ani1-2 RNAi did not disrupt cell cycle timing. By the way the description of the conclusion is explained in the text, I was expecting to see this data in the figure (lines 152-153).

Figure S1A shows that *ani-2* RNAi, on its own, does not affect cell cycle timing. To make this more clear, we changed this sentence to:

"RNAi of *ani-2* did not affect normal cell cycle progression in control, *pch-2*, or *mad-1* mutants (Figure S1A)"

To show that *ani-2* RNAi does not affect cell cycle timing when cells have monopolar spindles, we would compare wildtype sized *ani-2^{RNAi};zyg-1^{RNAi}* control AB cells to *zyg-1^{RNAi}* control AB cells. To address this, we added this to our analysis of binned data:

"To make these comparisons more clear, we binned our data. By our measurements, control AB cells ranged from 5 to 6 x 10^3 μm^3. Therefore, we classified AB cells greater than 5 x 10^3 μm^3 as wildtype sized. *ani-2^{RNAi};zyg-1^{RNAi}* AB cells that were wildtype sized exhibited mitotic delays while similarly sized *ani-2^{RNAi};zyg-1^{RNAi};pch-2* mutants produced no checkpoint response (Figure 1D). These data are consistent with what we have reported previously and report here for *zyg-1^{RNAi}* and *zyg-1^{RNAi};pch-2* AB cells (Nelson *et al.*, 2015 and Figure S4)."

2) More explanation is needed for the model. It is not clear why cmt-1 mutants would have more O-Mad2 available if CMT-1 is needed to convert C-Mad2 into O-Mad2. This would suggest that more C-Mad2 would be available.

We have changed this section to: "Given that p31^{comet} binds Mad2, specifically C-Mad2, throughout the cell cycle (Xia *et al.*, 2004; Date *et al.*, 2014) and that CMT-1 is required to maintain Mad2 protein levels (Nelson *et al.*, 2015), we hypothesize that CMT-1's binding of Mad2 plays two roles in *C. elegans*: to stabilize Mad2 and sequester it until required for checkpoint function. In the absence of CMT-1, more O-Mad2 is available despite the reduction in total protein levels, making PCH-2 partially dispensable and explaining the genetic suppression."

3) In the videos, it would be helpful to have a more detailed description to point out what we are supposed to take notice of. It is not clear when the onset of onset of cortical contractility occurs, so an arrow as to when this occurs would be helpful. Also, defining OCC in the figure legends would also be helpful.

We have indicated in the movie legends that OCC is visualized in cells with monopolar spindles as the formation of blebs at the cell membrane in AB cells and deformation of the cell membrane in P_1 cells and their timing in each movie.

4) There are a couple of places where the writing could be improved for clarity:
• In the abstract, the authors describe PCH-2 as remodeling the checkpoint effector Mad2 from an active conformation to an inactive one, but then the next sentences describe genetic data suggesting that PCH-2 is needed for spindle checkpoint activation. I think it is a hard transition to reconcile. Perhaps it would help to add

a sentence before the data about how you and others show that PCH-2 is needed for spindle checkpoint activation.

We have added this to the abstract:

“Having previously established that this function is required for spindle checkpoint activation, we demonstrate that in cells genetically manipulated to decrease in cell volume, PCH-2 is no longer required for the spindle checkpoint or recruitment of Mad2 at unattached kinetochores.”

• In the lines 190-195, the authors make two conclusions for their data in Figure 1. In the first conclusion, they state the results that pch-2 mutant AB cells have a robust spindle checkpoint as cells decrease in size, and their conclusion is that PCH-2 does not seem to affect spindle checkpoint silencing in C. elegans. While I agree with this conclusion, it did not seem to fit with the result just mentioned. The result mentioned suggest that PCH-2 has an important role in checkpoint activation in larger AB cells but not smaller AB cells. I think a third conclusion should be added in which the supporting data mentioned for lack of checkpoint silencing is that the pch-2 mutants have the same duration of spindle checkpoint activation as the control in the small embryos.

We have changed this to:

“Altogether, these data allow us to draw two important conclusions: First, the requirement for PCH-2 during spindle checkpoint activation is proportional to cell volume in AB cells with monopolar spindles. And second, since we observe similar mitotic timing in small *pch-2* mutant AB cells as in small control cells (Figure 1D), PCH-2 does not appear to affect spindle checkpoint silencing in *C. elegans*.”

• In lines 523-526, the authors state that PCH-2's characterized biochemical activity regulates the availability of O-MAD2. Can the authors be more specific about what the characterized biochemical activity is doing. Do you mean disassembling C-Mad2 from CMT-1 or is there something else? If this is the case, it is unclear why disassembling a checkpoint active C-MAD2 would allow greater checkpoint strength.

We have added this sentence to this section: “Given that *cmt-1* mutants exhibit decreased Mad2 levels (Nelson *et al.*, 2015), suggesting that CMT-1’s binding to C-Mad2 stabilizes the protein in *C. elegans*, we speculate that PCH-2 is specifically disassembling a C-Mad2/CMT-1 complex to generate this pool of O-Mad2.”

RE: Manuscript #E20-05-0310R
TITLE: "PCH-2/TRIP13 regulates spindle checkpoint strength"

Dear Needhi, All of the revisions are acceptable and properly addressed. I'd like to make one suggestion to make your work more broadly accessible is to slightly modify your title. I want more people to read your work. You can certainly take my suggestion or leave it. But I want the best for you and your lab.

From this currently:
"PCH-2TRIP13 regulates spindle checkpoint strength"

To this:
"A AAA-ATPase,PCH-2/TRIM13, regulates spindle checkpoint strength"

Sincerely,
Dr. Ahna Skop
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Bhalla:

Congratulations on the acceptance of your manuscript.

A PDF of your manuscript will be published on MBoC in Press, an early release version of the journal, within 10 days. The date your manuscript appears at www.molbiolcell.org/toc/mboc/0/0 is the official publication date. Your manuscript will also be scheduled for publication in the next available issue of MBoC.

Within approximately four weeks you will receive a PDF page proof of your article.

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We are pleased that you chose to publish your work in MBoC.

Sincerely,

Eric Baker
Journal Production Manager
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