A new layer of regulation of chromosomal passenger complex (CPC) translocation in budding yeast

Delaney Sherwin, Emily Gutierrez-Morton, Michael Bokros, Cory Haluska, and Yanchang Wang

Corresponding author(s): Yanchang Wang, Florida State University

Review Timeline:

Submission Date:	2023-02-22
Editorial Decision:	2023-03-27
Revision Received:	2023-06-26
Accepted:	2023-06-28

Editor-in-Chief: Matthew Welch

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 #E23-02-0063
TITLE: A new layer of regulation of chromosomal passenger complex (CPC) translocation in budding yeast

Dear Yanchang,
I am pleased to provide you with the constructive comments from two experts in the field. While they find the basic findings to be of high interest, both have significant suggestions to improve the impact of the conclusions and the clarity for the readership. In particular, reviewer 2 highlights the importance to substantiate the finding that Fin1 directly dephosphorylates Sli15, independent of Cdc14.
I look forward to reviewing a revised version that addresses these and the additional comments from the reviewers. Thank you for submitting this work to the Molecular Biology of the Cell.
Best,
Kerry

Sincerely,

Kerry Bloom
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Wang,

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 90 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):

In this manuscript, Sherwin et al. describe a new pathway that regulates the kinetochore to spindle translocation of the chromosomal passenger complex (CPC) during anaphase onset in budding yeast. The authors showed evidence that Fin1-PP1-mediated dephosphorylation of Sli15INCENP plays a role in the translocation of the CPC complex. Premature kinetochore targeting of Fin1 leads to the loss of Ipl1 localization at the kinetochores during metaphase. Moreover, in the absence of Cdc14 activity, forced localization of Fin1-PP1 to kinetochores results in partial translocation of CPC to the spindle. The authors went on to show that the phosphorylation of Sli15INCENP by Cdk and Ipl1 is required to correct tensionless attachments. The authors finally demonstrated that two distinct dephosphorylation events (Cdc14 and Fin1-PP1) regulate the spindle translocation dynamics of CPC, and abolishment of both pathways has an additive effect on CPC translocation dynamics. Overall, it is a thorough study that provides insights into a new regulatory pathway that controls CPC translocation in yeast, and the manuscript is well-written. However, there are certain results that require more validation to address the conclusions made in the manuscript.
Major comments :
The authors rely on the subtle phosphorylation shift patterns of Sli15 in WT and mutant backgrounds during different cell cycle stages. It would be more convincing if the authors could show the shift patterns clearly by using phos-tag gels or any other means possible. The authors may also include phosphorylation shift patterns for sli15-17A and sli15-6A across cell cycle stages. Since the combination of fin1-5A sli15-6A in the presence of Cik1 CC overexpression is additive, authors may also try to test to what extent Ipl1 localization to kinetochores is reduced in the fin1-5A sli15-6A mutant as it would further help strengthen the observation. Also, the authors may perform Sli15 western blot in the fin1-5A sli15-6A mutant to show the additive effect on reduced Sli15 phosphorylation levels.
Minor Comments:
The arrangement of the figure layout is confusing at times. The authors need to reformat with proper labels. The number of cells analyzed for quantification should be included in the figure legends for each graph. The authors should also crosscheck all the statistical analyses more carefully.
Page 4: Authors have mentioned that Bub1 kinase helps in the recruitment of CPC to centromeres via H3 phosphorylation. The authors should change it to haspin kinase instead of Bub1.
Page 5: Change the name from Bhm1 to Bmh1
Figure 1C: The authors mention that at 80 min there is a drop in phosphorylation of Sli15 in the text, but in figure 1C it is labeled as 60 min. The authors may also combine the Sli15 western with either Clb2 or Pds1 to define cell cycle stages.
Figure 1E: It will be helpful to compare FIN1, fin1-5A, cdc14-2 and cdc14-2 fin1-5A mutants to make the readers understand the relative contribution of these phosphatases towards Sli1 dephosphorylation in one panel.
Figure 2B: Representative image shows an equal number of cells showing a spindle-like localization of Ipl1 both in WT and sli15-17A. It should be changed.
Figure S2: The outlined cells do not look to be arrested at metaphase. The bud size is very small compared to the FIN1 panel. Also, the outline size is different for DIC and the rest of the panels. Have the authors quantified the budding index for these cells before doing the analyses?
Figure 3B: Provide corresponding DIC images or highlight the cell boundary in cdc14-2 + FIN1 background
Figure 4A: Do protein levels of Slk19-GFP change upon deletion of Fin1? Is there an increase in Slk19 intensity at the kinetochores suggesting it is recruited more at the kinetochore?
Figure 5B: Change sli15-17a to sli15-17A
Figure 5D: The text in the results says release at 36{degree sign}C, and the figure says 37{degree sign}C. The authors should be careful in mentioning these temperatures for release. Also, Pds1 levels appear to go down over time in the SLI15-myc mcd1-1 mutant as compared to that of mcd1-1 alone. Why is there a difference? The authors should explain.
Figure 7: The authors may include the spotting pictures for the mutants analyzed as shown in Figures 5A and 6A.
Overall, the statistical analyses should be done more rigorously. In a few cases, the difference between the control and test is quite significant, yet the authors show it to be significant only by '*.'

Reviewer #2 (Remarks to the Author):

The chromosomal passenger complex (CPC) is a protein complex involved in regulating mitosis and cytokinesis in eukaryotes. In yeast, it plays important roles in ensuring proper chromosome segregation and cytokinesis. Defects in CPC function can lead to chromosome mis-segregation and aneuploidy due to errors in the spindle assembly checkpoint (SAC) or in spindle formation. In this study, Sherwin et al. report on the phospho-regulation of the CPC protein Sli15. Sli15 is part of the Ipl1/Sli15 complex, which is present at kinetochores (KT) during most phases of the cell cycle. The complex phosphorylates key substrates to promote KT-microtubule (MT) interactions and chromosome biorientation. At KTs, Ipl1/Sli15 is also required to engage the SAC when KT-MT interactions lose tension. At metaphase to anaphase transition, the Cdc14-early anaphase release (FEAR) network actives the Cdc14 phosphatase. In turn, Cdc14 dephosphorylates Sli15 on cyclin-dependent kinase (CDK) phosphosites. This allows the translocation of the Ipl1/Sli15 complex from KT to the elongating mitotic spindle, where the complex stabilizes MT. This translocation also allows SAC silencing.

In the present work, the authors propose that Fin1-PP1 phosphatase regulates Sli15 KT-MT translocation by dephosphorylating Ipl1-phosphosites in Sli15, releasing Sli15 from KTs. Mutants in Fin1 that constitutively bind to KTs or Sli15 phospho-inhibitory mutants that escape Fin1-PP1 regulation cause premature Sli15 KT removal and defects in chromosome segregation and/or spindle stability. The authors suggest that Fin1-PP1 works in concert with Cdc14 to release Ipl1/Sli15 CPC complexes from kinetochores. Overall, the authors present a well-performed piece of work with interesting data that contributes to the understanding of mitotic control, particularly CPC regulation. Considering the conserved nature of the CPC complex, the model presented by the authors might be of interest to a broad audience. However, the direct phospho-regulation of Sli15 by Fin1-PP1 is less convincing, and the authors should conduct further analyses before publication to improve their findings.

Specific points:
1. The data presented in figure 1 does not convincingly show that Fin1-PP1 partially reverses Ipl1-phosphorylated sites of Sli15.
a. In Figure 1B, less phosphorylated Sli15 is equally observed in the ipl1-321 mutant at 25 and 37{degree sign}C. However, this not fit to the conclusion that Ipl1-321 mainly interferes with Sli15 phosphorylation at 37{degree sign}C (p. 6). The authors should clarify this point.
b. Figure 1C shows a time course experiment using WT and ipl1-321 cells. The conclusion is that Sli15 becomes hyperphosphorylated between 40 and 60 min, dropping at 80 min in WT, while hyperphosphorylation persists in ipl1-321. This is not obvious from the blots. In the side-by-side running of 40 and 60 min samples, it seems that Sli15 is less phosphorylated in ipl1-321 mutants in both time points. However, in the blots showing 0 - 160 min samples, there is no obvious difference between Sli15 in WT and ipl1-321 at 60 min - in these blots, Sli15 seems to become equally hyperphosphorylated at 60 min in both cell types.
c. Similarly, I don't see a clear decrease in Sli15 dephosphorylation in fin1-5A in figure 1D. This is more obvious in figure 1E in the cdc14-2 fin1-5A background but there is less Sli15 protein in the 40-100 min cdc14-2 fin1-5A compared to Cdc14-2 FIN1 samples. Therefore, I found the quantification of the 60 min bands shown in fig 1E not informative.
The authors should improve these data. One problem in this type of experiments is the synchrony of cultures. Perhaps the authors should consider comparing Sli15 phospho-patterns using other types of synchronisations (e.g. Gal1-CDC20 depletion for metaphase arrest instead of alpha-factor arrest/release; or alternative approaches).
2. In Figure 1, the authors only show Pgk1 as a loading control but no other biochemical cell cycle marker as a quality control for the synchronization. The percentage of large-budded cells is informative, but does not accurately reflect when cells exactly enter mitosis and undergo the metaphase-anaphase transition. As ipl1-321 and cdc14-2 might transit slowly into mitosis, the comparison of Sli15 in cells at the same cell cycle stage is to my knowledge very important. Therefore, more markers (such as Clb2, Pds1, Sic1) and even spindle length should be included.
3. If possible, it would be important to have other biochemical evidence for Sli15 dephosphorylation by Fin1-PP1A (co-IP, in vitro?). Or any other strong evidence that Fin1-PP1 is directly involved in Sli15 regulation. For ex., does Sli15-17D/E rescues the phenotype of Fin1-5A or ipl1-321 cells but not Cdc14-2 cells?
4. Figure 4 would benefit from a different type of representation of the data. In fig. 4A, the length of spindles shown in WT and fin1Δ cells is different, making it hard to compare Slk19 localization. For this type of analysis, it would be good to see Slk19 spindle/midzone localization as a function of spindle size and not time, as currently depicted.
5. Spindle breakage is not clear in the images shown in Fig 4C and 4D. In these images, it would be good to see the phase contrast images, as cells seem to be larger in cdc15-2 fin1Δ in comparison to cdc15-2 cells.
6. I am confused with the Pds1 levels shown for mcd1-1 and mcd1-1 Sli15-myc in Fig. 5D (blots). In both strains, Pds1 levels should be identical, but Pds1 degradation is not obvious in mcd1-1, while it peaks between 40 and 80 min in mcd1-1 Sli15-myc cells. Please clarify.
7. The graph shown in Fig. 5D to the right (ratio of Pds1/Pgk1) is not clearly described in the text.
8. It is not clear what the authors mean about combining data from figs. 5, 6 and 7 to make the graph in fig. 7C. Does it mean that experiments were performed at different times for each particular mutant? Due to high variation in such experiments, all strains should be analysed together.

Minor points.
1. It would be good to refer to the figure as soon as the authors start to describe the data (and not only at the end of data description).
2. There are a couple of typos throughout the text.

Response for reviewers' comments

We appreciate the comments from the editor and each reviewer. We believe that the quality of this research work has been improved significantly by addressing these comments. Below is the point-by-point response to these comments.

Comments from the Editor: *I am pleased to provide you with the constructive comments from two experts in the field. While they find the basic findings to be of high interest, both have significant suggestions to improve the impact of the conclusions and the clarity for the readership. In particular, reviewer 2 highlights the importance to substantiate the finding that Fin1 directly dephosphorylates Sli15, independent of Cdc14. I look forward to reviewing a revised version that addresses these and the additional comments from the reviewers. Thank you for submitting this work to the Molecular Biology of the Cell.*

Response: We appreciate the constructive comments from you and both reviewers. We have performed experiments as suggested by the reviewers and many new results are included in the revised version. To better examine Sli15 phosphorylation, we used Phos-tag SDS-PAGE for an improved separation of phosphorylated species of Sli15 protein as suggested. Most of the results in the previous Figure 1 are replaced by new results from Phos-tag SDS-PAGE (**Fig. 1C, D, E, and F**), which we believe significantly strengthen our findings that Fin1-PP1 contributes to Sli15 dephosphorylation (Reviewer #1 point 1, Reviewer #2 point 1). Although we could not perform an *in vitro* phosphatase reaction to demonstrate that Fin1-PP1 dephosphorylates Sli15 directly (the reason is explained, Reviewer #2 point 7), in the revised version we presented much stronger evidence showing Fin1-dependent Sli15 dephosphorylation even in the absence of Cdc14 activity. In addition, we presented more evidence showing an additive effect of *fin1-5A* and *sli15-6A* on Ipl1 delocalization from kinetochores (**Fig. 7D**). We have also tried hard to address all other concerns from the reviewers. We believe that the quality of this manuscript is improved drastically because of these constructive suggestions. For the review process, sections with major changes are marked as blue.

Reviewer 1:

Major comments:

1) *The authors rely on the subtle phosphorylation shift patterns of Sli15 in WT and mutant backgrounds during different cell cycle stages. It would be more convincing if the authors could show the shift patterns clearly by using phos-tag gels or any other means possible. The authors may also include phosphorylation shift patterns for sli15-17A and sli15-6A across cell cycle stages.*

Response:

We highly appreciate this constructive suggestion. Indeed, our new Phos-tag results significantly improve the separation of phosphorylated Sli15 protein species. Using Phos-tag SDS-PAGE, we first showed the cell cycle regulated Sli15 dephosphorylation, which concurs with Pds1 degradation (**Fig. 1C**). In addition, less phosphorylated Sli15 species were detected in metaphase cells with either compromised Ipl1 activity (**Fig. 1D**) or premature Fin1 kinetochore localization (*fin1-5A*) (**Fig. 1E**). We further found that expression of Fin1-5A induced premature Sli15 dephosphorylation even in the absence of Cdc14 activity (**Fig. 1F**). Finally, we showed that expression of Fin1-5A caused dephosphorylation of Sli15-6A-13myc (CDK site-deficient mutant), but not Sli15-17A-13myc (Ipl1 site-deficient mutant) (**Fig. 7E**). All these new data strengthen the notion that

kinetochore localization of Fin1-PP1 contributes to Sli15 dephosphorylation, likely reversing Ipl1-mediated phosphorylation.

2) *Since the combination of fin1-5A sli15-6A in the presence of Cik1 CC overexpression is additive, authors may also try to test to what extent Ipl1 localization to kinetochores is reduced in the fin1-5A sli15-6A mutant as it would further help strengthen the observation. Also, the authors may perform Sli15 western blot in the fin1-5A sli15-6A mutant to show the additive effect on reduced Sli15 phosphorylation levels.*

   Response: Thanks for these constructive ideas. As suggested, we analyzed kinetochore localization of Ipl1 in *sli15-6A* and *sli15-17A* mutant cells expressing either *FIN1* or *fin1-5A*. We observed a significant increase of Ipl1 kinetochore delocalization in *sli15-6A fin1-5A* cells arrested in metaphase compared to *sli15-6A* cells (**Fig. 7D**). However, *sli15-17A fin1-5A* cells did not show a clear additive effect on Ipl1 kinetochore delocalization. In addition, we constructed *sli15-6A-13myc* and *sli15-17A-13myc* strains and performed Phos-tag SDS-PAGE. The results show that Fin1-5A expression reduces phosphorylation of Sli15-6A-13myc (CDK site deficient), but not Sli15-17A-13myc (Ipl1 site deficient) (**Fig. 7E**). Therefore, all these new data further support the idea that Fin1-PP1 reverses Sli15 dephosphorylation imposed by Ipl1, but not by CDK.

Minor comments:

1) *The arrangement of the figure layout is confusing at times. The authors need to reformat with proper labels.*

   Response: Great suggestion! We have updated the arrangement of the figures.

2) *The number of cells analyzed for quantification should be included in the figure legends for each graph. The authors should also crosscheck all the statistical analyses more carefully.*

   Response: We agree. In the revised version, we have included the number of cells analyzed for quantification in the figure legends. Additionally, we have performed the statistical analyses more rigorously.

3) *Page 4: Authors have mentioned that Bub1 kinase helps in the recruitment of CPC to centromeres via H3 phosphorylation. The authors should change it to haspin kinase instead of Bub1.*

   Response: Sorry about the mistake. We have made the change to haspin kinase in the revised text and the associated citations.

4) *Page 5: Change the name from Bhm1 to Bmh1.*

   Response: Thank you for catching this typo. We have changed it to Bmh1 in the revised version.

5) *Figure 1C: The authors mention that at 80 min there is a drop in phosphorylation of Sli15 in the text, but in figure 1C it is labeled as 60 min. The authors may also combine the Sli15 western with either Clb2 or Pds1 to define cell cycle stages. (add Pds1 in a separated figure?)*

Response. We agree. To address this, we have modified **Figure 1C** to analyze Sli15 phosphorylation using Phos-tag SDS-PAGE in parallel with Pds1 protein levels to define cell cycle stages. It is clear that Sli15 dephosphorylation occurs after Pds1 degradation (80 min) during the cell cycle.

6) *Figure 1E: It will be helpful to compare FIN1, fin1-5A, cdc14-2 and cdc14-2 fin1-5A mutants to make the readers understand the relative contribution of these phosphatases towards Sli15 dephosphorylation in one panel.*

Response: As suggested, we repeated the experiment in **Figure 1E** in the original manuscript using Phos-tag SDS-PAGE to visualize the relative contribution of Fin1-PP1 and Cdc14 towards Sli15 dephosphorylation (now **Fig. 1F**). To this end, we monitored Sli15 phosphorylation in *FIN1* and *fin1-5A* cells in *cdc14-2* background after G_1 release into the nonpermissive temperature. After G1 release for 40 min, we found clearly decreased Sli15 phosphorylation in *fin1-5A cdc14-2* cells compared to *cdc14-2* alone. Our new result with Phos-tag is much more convincing due to the enhanced resolution of phosphorylated Sli15 protein species. The new result further strengthens the notion that premature Fin1-PP1 localization to the kinetochore (Fin1-5A) causes Sli15 dephosphorylation in a Cdc14-independent manner.

7) *Representative image shows an equal number of cells showing a spindle-like localization of Ipl1 both in WT and sli15-17A. It should be changed.*

Response: Great point. We have updated this panel to be more representative.

8) *Figure S2: The outlined cells do not look to be arrested at metaphase. The bud size is very small compared to the FIN1 panel. Also, the outline size is different for DIC and the rest of the panels. Have the authors quantified the budding index for these cells before doing the analyses?*

Response: In our revised version, we updated this figure panel to be more representative. We have checked the budding index at 100 minutes following G_1 release and found that cells from both groups, *FIN1* and *fin1-5A-AA*, were 79% and 74% large budded, respectively.

9) *Figure 3B: Provide corresponding DIC images or highlight the cell boundary in cdc14-2 + FIN1 background*

Response: Thank you for the suggestion. In our updated version, we have highlighted the cell boundary in *cdc14-2 + FIN1/fin1-5A*.

10) *Figure 4A: Do protein levels of Slk19-GFP change upon deletion of Fin1? Is there an increase in Slk19 intensity at the kinetochores suggesting it is recruited more at the kinetochore?*

Response: We repeated this experiment using asynchronous cells and examined the Slk19 midzone localization in WT and *fin1Δ* cells in anaphase (with an elongated spindle). Thus, we have modified **Figure 4A** to monitor Slk19 midzone localization as a function of spindle length and not time. After repeating this experiment in triplicate, we did find decreased spindle midzone localization of Slk19-GFP in *fin1*Δ compared to WT cells, which is consistent with our data in the previous version. However, we did not see a substantial difference in Slk19-GFP intensity between WT and *fin1*Δ cells. We also used asynchronous cells to analyze Ase1 spindle midzone localization in WT and *fin1Δ* cells. The results in **Figure 4A/B** in previous submission are replaced with these new ones.

11) *Figure 5B: Change sli15-17a to sli15-17A*

Response: Thank you for catching this typo. It has been corrected.

12) *Figure 5D: The text in the results says release at 36^{o}C, and the figure says 37^{o}C. The authors should be careful in mentioning these temperatures for release. Also, Pds1 levels appear to go down over time in the SLI15-myc mcd1-1 mutant as compared to that of mcd1-1 alone. Why is there a difference? The authors should explain.*

Response: We apologize for making this mistake in the figure panel. In our revised manuscript, we fixed this issue to show that the release is at 36°C. We have removed *mcd1-1* alone from the panel (**Fig. 5D**), as *mcd1-1 SLI15-myc* is most similar in genotype to *mcd1-1 sli15-17A-13myc*. Pds1 levels in *mcd1-1 sli15-17A-13myc* cells showed an appreciable decrease in comparison to *mcd1-1 SLI15-myc13*. This difference is reproducible as we repeated this experiment in triplicate and found similar results each time. Now, quantitative results are shown.

13) *Figure 7: The authors may include the spotting pictures for the mutants analyzed as shown in Figures 5A and 6A.*

Response: Great suggestion. We have included the spotting assays for the mutants in **Figure S4**.

14) *Overall, the statistical analyses should be done more rigorously. In a few cases, the difference between the control and test is quite significant, yet the authors show it to be significant only by '*.'*

Response: We appreciate your comment! In our original manuscript, for most experiments, we performed a non-parametric Wilcoxon rank sum test using GraphPad Prism software to determine *p* values. We chose this statistical analysis because it makes no assumption about the distribution of a population. However, this statistical analysis cannot produce higher significance than $p < 0.05$ (*) for groups with seven or fewer replicates, even when the difference between the two groups is quite significant. For this reason, we first performed Shapiro-Wilk tests to ensure that the data follow a normal distribution before performing t-tests.

Reviewer 2:

Major comments:

1) *The data presented in figure 1 does not convincingly show that Fin1-PP1 partially reverses Ipl1-phosphorylated sites of Sli15.*

Response: We agree. The first reviewer expressed a similar concern. To substantiate the notion that Fin1-PP1 reverses Ipl1-mediated phosphorylation of Sli15, we have included new results using Phos-tag SDS-PAGE to better examine Sli15 phosphorylation. The following new Phos-tag results strengthen the notion that Fin1-PP1 likely reverses Ipl1-phosphorylated sites of Sli15. First, we found that expression of Fin1-5A caused dephosphorylation of Sli15-6A-myc (CDK site deficient), but not Sli15-17A-myc (Ipl1 site-deficient) (**Fig. 7E**). Moreover, we showed that expression of Fin1-5A induced significant Sli15 dephosphorylation in *cdc14*-2 mutants (**Fig. 1F**), and previous works show that Cdc14 specifically dephosphorylates CDK substrates. These results indicate that Fin1-PP1 likely reverses Ipl1-imposed Sli15 phosphorylation, which is independent of Cdc14 that specially reverses CDK-imposed Sli15 phosphorylation.

2) *In Figure 1B, less phosphorylated Sli15 is equally observed in the ipl1-321 mutant at 25 and 37°C. However, this not fit to the conclusion that Ipl1-321 mainly interferes with Sli15 phosphorylation at 37°C (p. 6). The authors should clarify this point.*

Response: We appreciate this comment. Using Phos-tag SDS-PAGE, we also detected less phosphorylated Sli15 in *ipl1-321* mutants arrested at metaphase (P_{GAL}*CDC20*) at 25°C and 37°C (**Fig. 1D**). One possibility is that mutated Ipl1-321 kinase has reduced catalytic activity even at the permissive temperature 25°C. A previous work shows impaired kinetochore assembly and kinetochore-microtubule attachments in *ipl1-321* mutants at 25°C (*Biggins et al. 1999 Genes Dev*). We previously found defective checkpoint in response to tensionless kinetochore attachment in *ipl1-321* mutant cells at 25°C (*Jin et al. 2012 PLoS Genetics*). We have revised the manuscript to clarify this point.

3) *Figure 1C shows a time course experiment using WT and ipl1-321 cells. The conclusion is that Sli15 becomes hyperphosphorylated between 40 and 60 min, dropping at 80 min in WT, while hyperphosphorylation persists in ipl1-321. This is not obvious from the blots. In the side-by-side running of 40 and 60 min samples, it seems that Sli15 is less phosphorylated in ipl1-321 mutants in both time points. However, in the blots showing 0 - 160 min samples, there is no obvious difference between Sli15 in WT and ipl1-321 at 60 min - in these blots, Sli15 seems to become equally hyperphosphorylated at 60 min in both cell types.*

Response: We agree that the evidence for this notion from the previous submission is not strong. As mentioned above, we used Phos-tag gel to compare Sli15 phosphorylation in WT and *ipl1-321* cells arrested at metaphase (P_{GAL}*CDC20*), and the difference in Sli15 phosphorylation between WT and *ipl1-321* is much more convincing (**Fig. 1D**). The SDS-PAGE result of Sli15 phosphorylation in WT and *ipl1-321* cells during the cell cycle from previous submission (**Fig. 1C**) is removed in the revised manuscript.

4) *Similarly, I don't see a clear decrease in Sli15 dephosphorylation in fin1-5A in figure 1D. This is more obvious in figure 1E in the cdc14-2 fin1-5A background but there is less Sli15 protein in the 40-100 min cdc14-2 fin1-5A compared to Cdc14-2 FIN1 samples. Therefore, I found the quantification of the 60 min bands shown in fig 1E not informative.*

Response: Yes, we agree. Our data relied on subtle phosphorylation shift patterns from regular western blots in the previous submission. As suggested, we incorporated Phos-tag

results to show the decrease in Sli15 phosphorylation in *fin1-5A* cells. The cell cycle kinetics of Sli15 phosphorylation in WT and *fin1-5A* in the previous submission (**Fig. 1D**) was removed in this version. Instead, we compared Sli15 phosphorylation using Phos-tag gel in metaphase arrested (P_{GAL}*CDC20*) cells expressing *FIN1*, *fin1-5A*, and *fina-5A-AA* . The new result in **Figure 1E** shows a clear decrease in Sli15 phosphorylation in cells expressing fin1-5A. However, the abolishment of Fin1-PP1 binding (Fin1-5A-AA) restores efficient Sli15 phosphorylation. Although Fin1-5A expression clearly decreases Sli15 phosphorylation in *cdc14-2* cells in the previous submission, in the revised version, we also include results using Phos-tag gel in the revised version (**Fig. 1F**). The new results are more convincing to show the decreased Sli15 phosphorylation in *cdc14-2* cells expressing Fin1-5A.

5) *The authors should improve these data. One problem in this type of experiments is the synchrony of cultures. Perhaps the authors should consider comparing Sli15 phospho-patterns using other types of synchronisations (e.g. Gal1-CDC20 depletion for metaphase arrest instead of alpha-factor arrest/release; or alternative approaches).*

Response: We appreciate the constructive feedback. As suggested, we utilized P_{GAL}*CDC20* strains to deplete Cdc20 for metaphase arrest in **Figures 1D** and **1E.** We then compared Sli15 phosphorylation in WT, *ipl1-321, fin1-5A,* and *fin1-5A-AA* mutants using Phos-tag SDS-PAGE. Notably, both *ipl1-321* and *fin1-5A* mutant cells showed decreased Sli15 phosphorylation with a similar pattern.

6) *In Figure 1, the authors only show Pgk1 as a loading control but no other biochemical cell cycle marker as a quality control for the synchronization. The percentage of large-budded cells is informative, but does not accurately reflect when cells exactly enter mitosis and undergo the metaphase-anaphase transition. As ipl1-321 and cdc14-2 might transit slowly into mitosis, the comparison of Sli15 in cells at the same cell cycle stage is to my knowledge very important. Therefore, more markers (such as Clb2, Pds1, Sic1) and even spindle length should be included.*

Response: We appreciate this suggestion. We agree that it is important to clarify the cell cycle stage when Sli15 becomes dephosphorylated. In the revised version, we combined the Sli15 western blot (Phos-tag) with Pds1 following G_1 release **(Fig. 1C**). It is clear that Sli15 dephosphorylation occurs at 80 min after G1 release when Pds1 protein level drops, indicating that Sli15 is dephosphorylated after anaphase entry.

We also agree that the comparison of Sli15 phosphorylation in WT and mutant cells at the same cell cycle stage is important, thus we used *CDC20* depletion (P_{GAL}*CDC20*) for metaphase arrest as suggested, and then compared the impacts of mutations in *IPL1* and *FIN1* genes on Sli15 phosphorylation (**Fig. 1D** and **E**). Significant decrease of Sli15 phosphorylation was observed in *ipl1-321* and *fin1-5A* mutant cells arrested at metaphase by Cdc20 depletion.

7) *If possible, it would be important to have other biochemical evidence for Sli15 dephosphorylation by Fin1-PP1A (co-IP, in vitro?). Or any other strong evidence that Fin1-PP1 is directly involved in Sli15 regulation. For ex., does Sli15-17D/E rescues the phenotype of Fin1-5A or ipl1-321 cells but not Cdc14-2 cells?*

Response: Thank you for the suggestion. We tried performing an *in vitro* phosphatase assay to measure Sli15 dephosphorylation by Fin1-PP1 either as a function of time or Fin1-PP1 concentration. We performed this experiment using IPed Sli15 and Fin1 from yeast

cells. Unfortunately, several attempts at purifying these proteins from yeast cells resulted in multiple degradation products that made the resulting *in vitro* phosphatase assays difficult to interpret.

However, our revised manuscript includes a new set of results using Phos-tag SDS-PAGE, providing stronger evidence to support the idea that Fin1-PP1 dephosphorylates Sli15 imposed by Ipl1, but not by CDK. First, *ipl1-321* and *fin1-5A* mutants arrested in metaphase (*P_{GAL}CDC20*) exhibited decreased Sli15 phosphorylation in a similar pattern (**Fig. 1D** and **E**). Second, expression of Fin1-5A reduces Sli15 phosphorylation, but this effect is abolished in cells expressing Fin1-5A-AA that lacks Fin1-PP1 binding (**Fig. 1E**). In addition, expression of Fin1-5A reduces the phosphorylation of Sli15-6A (CDK site deficient), but not Sli15-17A (Ipl1 site deficient) (**Fig. 7E**). These are stronger evidence supporting the idea that Fin1-PP1 reverses Sli15 phosphorylation imposed by Ipl1.

8) *Figure 4 would benefit from a different type of representation of the data. In fig. 4A, the length of spindles shown in WT and fin1Δ cells is different, making it hard to compare Slk19 localization. For this type of analysis, it would be good to see Slk19 spindle/midzone localization as a function of spindle size and not time, as currently depicted.*

Response: Great suggestion! As suggested, In **Figure 4A**, we examined Slk19 midzone localization as a function of spindle length. We performed the experiment using asynchronous cells and measured the percentage of cells with an elongated spindle that showed Slk19 midzone localization. Using a similar protocol, we repeated the experiment to assess the spindle midzone localization of Ase1 (**Fig. 4B**).

9) *Spindle breakage is not clear in the images shown in Fig 4C and 4D. In these images, it would be good to see the phase contrast images, as cells seem to be larger in cdc15-2 fin1Δ in comparison to cdc15-2 cells.*

Response: Thank you for this comment. We have highlighted the cell boundary in **Figure 4C**. We have also repeated the experiment in **Figure 4D** and new images are shown with clear cell boundary and spindle structure.

10) *I am confused with the Pds1 levels shown for mcd1-1 and mcd1-1 Sli15-myc in Fig. 5D (blots). In both strains, Pds1 levels should be identical, but Pds1 degradation is not obvious in mcd1-1, while it peaks between 40 and 80 min in mcd1-1 Sli15-myc cells. Please clarify.*

Response: We apologize for this confusion. In the revised version, we just compared the Pds1 protein levels in *mcd1-1 SLI15-my*s and *mcd1-1 sli15-17A-myc* cells, since these two strains have the same genotype except *SLI15* gene (**Fig. 5D**). In addition, we repeated this experiment in triplicate for statistical analysis of Pds1 protein levels during the cell cycle. Pds1 levels in *mcd1-1 sli15-17A-myc13* cells showed an appreciable decrease in later time points in comparison to *mcd1-1 SLI15-myc13*.

11) *The graph shown in Fig. 5D to the right (ratio of Pds1/Pgk1) is not clearly described in the text.*

Response: We have fixed this in the revised version.

12) It is not clear what the authors mean about combining data from figs. 5, 6 and 7 to make the graph in fig. 7C. Does it mean that experiments were performed at different times for each particular mutant? Due to high variation in such experiments, all strains should be analysed together.

Response: We apologize for the confusion. The experiments in **Figure 7** were indeed performed together for each mutant and this section is reworded in the current version. In addition, in the revised version, we further analyzed the Ipl1 localization in *sli15-6A* and *sli15-17A* cells expressing *FIN1* or *fin1-5A* as suggested by the other reviewer (**Fig. 7D**). The new results support an additive effect between *sli15-6A* and *fin1-5A* on the kinetochore delocalization of Ipl1, which is consistent with the increased viability loss in *sli15-6A fin1-5A* cells after Cik1-CC overexpression (**Fig. 7C**).

Minor comments:

1) It would be good to refer to the figure as soon as the authors start to describe the data (and not only at the end of data description).

Response: Thank you for the suggestion! In the revised version, we have referred to the figures at the beginning of each description.

2) There are a couple of typos throughout the text.

Response: Thank you. We have carefully checked for typos in the revised version.

RE: Manuscript #E23-02-0063R
TITLE: "A new layer of regulation of chromosomal passenger complex (CPC) translocation in budding yeast"

Dear Yangchang,

Thank you for your thoughtful and thorough response to the reviewers comments. I am pleased to inform you that the manuscript is now suitable for publication in the Molecular Biology of the Cell. Thank you for this interesting submission.
Best,
kerry

Kerry Bloom
Monitoring Editor
Molecular Biology of the Cell

Dear Dr. Wang:

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.

MBoC offers the option to publish your paper with immediate open access. Open access can increase the discoverability and usability of your research. If you would like to publish your paper with immediate open access but did not select this option during initial submission, please contact the MBoC Editorial Office (mbc@ascb.org).

We invite you to submit images related to your accepted manuscript to be considered for the journal cover. Please contact mboc@ascb.org to learn how to submit candidate cover images.

Authors of Articles and Brief Communications are also encouraged to create a short video abstract to accompany their article when it is published. These video abstracts, known as Science Sketches, are up to 2 minutes long and will be published on YouTube and then embedded in the article abstract. Science Sketch Editors on the MBoC Editorial Board will provide guidance as you prepare your video. Information about how to prepare and submit a video abstract is available at www.molbiolcell.org/science-sketches. Please contact mboc@ascb.org if you are interested in creating a Science Sketch.

We look forward to publishing your paper in MBoC.

Sincerely,

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