Journal of Cell Science

Review COMMONS

Altered cohesin dynamics and histone H3K9 modifications contribute to mitotic defects in the *cbf11Δ* lipid metabolism mutant

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DOI: 10.1242/jcs.261265

Editor: Maria Carmo-Fonseca

Review timeline

Submission to Review Commons:	18 October 2022
Submission to Journal of Cell Science:	20 April 2023
Accepted:	8 May 2023

Reviewer 1

Evidence, reproducibility and clarity

Summary:

The manuscript by Vishwanatha et al. presents findings on the fission yeast transcription factor Cbf11, which is involved in regulating lipid synthesis. Changes in lipid metabolism often have detrimental effects on nuclear division (evidenced by the high percentage of cut phenotypes among strains with altered lipid content). Here the authors show that cbf11 deletion strains produce additional phenotypes such as changes to cohesion dynamics and altered chromatin modification within centromeric regions, in turn perhaps affecting microtubule attachment and proper chromosome distributions. This hypothesis is supported by the authors' finding of epistatic effects between cbf11 and cohesin loading and unloading.

Major comments:

While the evidence presented supports the hypothesis of altered cohesin loading as a major driver of observed mitotic defects, changes in the NE surface area are likely to also contribute to the phenotypes even in pre-anaphase stages. Did the authors test any double deletions with regulators involved in decreasing lipid content (e.g. spo7, nem1, ned1) to counteract the role of Cbf11? This could be useful in assessing the relative contribution of cohesion dynamics and histone modifications.

A possible role of physical constraints dictated by the NE was already mentioned by the authors in the context of spindle bending and decreased elongation rates and some preliminary experimental data on this would be appreciated. Generation of strains, acquisition of some timelapses, and quantification of spindle elongation rate/buckling frequency should be feasible in a reasonable time frame.

The authors report mRNA levels of the centromere flanking genes per1 and sdh1 to be increased by 1.5x and decreased by 2x in comparison to WT. Could the authors elaborate on whether this is an expected trend? Kaufmann et al., 2010 reported low transcription of per1 when the surrounding regions are predominantly acetylated. Fig. 4A suggests a slight increase of H3K9ac at per1 and a decrease of transcription would be conceivable.

Fig. 3B indicates a catastrophic mitosis percentage of roughly 9.5% in cbf11Δ while in Fig. 1C 4% of all cells, or ~31% of all mitotic events, is noted as abnormal. Could the authors clarify this discrepancy? Since Fig. 1 utilises time course data of 333 cells (please specify the number of analysed cells also in the legend), would the authors expect this data to be more trustworthy

when compared to images of fixed cells? What were the criteria to assign divisions as catastrophic in fixed cells and which features were utilised to identify the 400 cells as mitotic?

Minor comments:
Previous literature is, to the best of our knowledge, sufficiently referenced. The text is largely clear (some exceptions within the methods section will be elaborated on below). The figures, however, would benefit from graph titles and some minor formatting changes.

- Figures:
- Fig. 1: Specify the number of cells analysed in C within the legend as well. For B, please use colourblind-friendly schemes - especially since images are shown as merges only. The example of the "cut" phenotype appears small and crowded by surrounding cells. Especially the latter might affect mitotic fidelity. Under the assumption that this did not affect quantifications (WT seem fine) a less crowded cell would present a nicer example.
- Fig. 3: Images shown in A add little benefit in their current form. What is the takeaway for the reader? Indicating that images represent DAPI staining and pointing out cells of interest with arrows/symbols would be helpful. The example shown for cbf11 appears to be dimmer in comparison and cell morphology is hard to interpret. C feels misplaced in this figure and a title could improve readability.
- Fig. 4: Graph titles needed, figure might work better in portrait
- Text:
- Mention median duration of mitosis in cbf11Δ (Fig. 2E) in text since WT is already noted;
- Discussion, third paragraph: "TBZ [REF] and are prone to chromosome loss [...]". I assume this referred to minichromosome loss or have changes in ploidy/chromosome segregation been quantified?

- Methods, Microscopy and image analysis:
How were fixed cells imaged (glass bottom dishes, plated on lectin, mounted on slides)? Specify the CellR as widefield and provide details of the objective used (immersion and NA) Elaborate on "manual evaluation of microscopic images"
For live cell microscopy, what was the estimated final density of cells within the 5 µl resuspension?
What is meant by measuring the maximum section of plotted profiles? Is this the maximum distance of Hht1 signals within the entire time-lapse?
Was spindle length quantified the same way? Methods, ChIP-qPCR:
It is not clear which strains were used, this can only be guessed by the use of a GFP antibody suggesting GFP tagged chromatin to be precipitated. For people with expertise outside of ChIP assays, this should be specified

Significance

Describe the nature and significance of the advance (e.g. conceptual, technical, clinical) for the field.

This manuscript presents a novel role for a transcription factor, one typically implicated in lipid metabolism, in chromatin modification and cohesin dynamics, with the possibility of this representing a more conserved process across ascomycetes. The mechanism of cbf11 regulation remains to be determined.

Place the work in the context of the existing literature (provide references, where appropriate).

This work helps link two bodies of work related to cell division that are usually considered in isolation, the regulation of lipid dynamics and the control of chromatin dynamics and cohesion. Some comparisons to phenotypes in closely related species would have helped provide a broader context (such as Yam et al., 2011, where the spindle morphologies in S. japonicus and response to cerulenin treatment might be of relevance to the work presented here).

State what audience might be interested in and influenced by the reported findings.

Molecular and cellular biologists with interests in nuclear remodelling, lipid metabolism, kinetochore assembly.

Define your field of expertise with a few keywords to help the authors contextualize your point of view. Indicate if there are any parts of the paper that you do not have sufficient expertise to evaluate.

Fission yeast biology, nuclear remodelling, microscopy. We are not qualified to make in- depth comments on the soundness of ChIP-Seq and ChIP-qPCR experiments.

Reviewer 2

Evidence, reproducibility and clarity

This manuscript describes detailed mechanisms by which the cbf11 deletion showed the phenotype. They found that the cbf11 deletion altered pericentromeric chromatin states such as the level of cohesin and hypermethylation.

In general, their results are interesting and provide important insights into the relationship between lipid metabolism and chromosome segregation. The presented data are valuable for the community, but the authors should carefully re-assess their data.

Major comments:

1. Statistical analyses in some of the Fig.3B, 3C, 4B and S2 seem to be somewhat weird because p-values are too small for such a small number of experiments (three independent experiments) with large standard deviations. Please show all the data points in Fig. 2C-E, and provide raw values as a supplementary table for assessment of the data.

2. Pages 5-6: As for Fig. 4, the data is difficult to interpret because the trends of the ChIP- seq pattern of H3K9me2 between replicates look different: replicate 2 shows an increase of H3K9me2 signal, while replicate 1 shows almost no difference or weak if any. In such a case, the authors should repeat ChIP-seq one more time and confirm hypermethylation at these regions or confirm it by ChIP-qPCR. Assuming that the pericentromeric regions are hypermethylated by cbf11 deletion, it is still unclear why the transcription from only dh, but not dg, regions increased although their ChIP-seq data indicated both dh/dg regions were hypermethylated. A similar question arises to the expression of per1 and sdh1. Both K9Ac and K9me2 modifications seem to unchange at both per1 and sdh1 loci, whereas the expression levels of these loci changed in the opposite direction. These results suggest that the transcription levels of the centromeric region are independent of their histone modification states.

3. A key question of this study is to understand the relationship between lipid metabolism and chromosome structures. However, the results presented are not enough to address this question. I request to distinguish whether the defects on pericentromeric regions are mediated by lipid metabolism or direct effect by cbf11 deletion. Cbf11 is a transcription factor and can directly bind to DNA, thereby there is a possibility that Cbf11 directly modulates the pericentromeric chromatin state without regulating lipid metabolism. This question can probably be addressed. As the authors have shown in their previous study (Prevorovsky et al., 2016), overexpression of cut6, which encodes acetyl coenzyme A carboxylase and is a target of cbf11, can bypass nuclear defects. If the overexpression of cut6 restores alteration on pericentromeric regions such as cohesin enrichment and hypermethylation, it suggests
the defects are a secondary effect of the decrease of phospholipid biosynthesis.

Minor comments:

4. Figure 3C: The legend says, "Values represent means + SD from 3 independent experiments". It meant "means {plus minus} SD"?

5. The relationship between phospholipid synthesis and mitotic fidelity is now discussed in the bioRxiv paper (https://doi.org/10.1101/2022.06.01.494365). It would be nice to discuss this paper.

Significance

Faithful chromosome segregation into daughter cells is crucial for cell proliferation. The authors previously reported that the deletion of cbf11, a transcription factor that regulates lipid metabolism genes, causes "cut (cell untimely torn)" phenotype (Prevorovsky et al., 2015; Prevorovsky et al., 2016). In this report, they examined detailed mechanisms by which the cbf11 deletion showed the phenotype, and found that the cbf11 deletion altered pericentromeric chromatin states such as the level of cohesin and hypermethylation. In general, their results are interesting and provide important insights into the relationship between lipid metabolism and chromosome segregation. The presented data are valuable for the community of basic science in the fields of chromosome biology and cell biology.

We are cell biologists working on chromosomes and the cell nucleus.

Reviewer 3

Evidence, reproducibility and clarity

The Vishwanatha et al. manuscript examined the nature of the mitotic defect in cbf11 deletion cells. cbf11+ encodes a CSL transcription factor that regulates lipid metabolism genes in S. pombe. Loss of cbf11+ was previously shown to have a "cut" phenotype presumably due in part to aberrant regulation of its target gene cut6+ which encodes-acetyl CoA/biotin carboxylase involved in fatty acid biosynthesis (Zach et al. 2018). The authors hypothesized that the mitotic defect exhibited as chromosome missegregation in cbf11 deletion cells may be caused by alterations in cohesin occupancy and H3K9 methylation in centromeres. Cohesin occupancy was slightly higher in centromeric dh and dg repeats in the cbf11 mutant and loss of the cohesin-loader gene wpl1+ appeared to suppress the mitotic defect. The authors also showed by ChIP-Seq that H3K9 methylation was higher in the centromeric regions, as well as increased minichromosomal loss in the cbf11 mutant.

The discovery of increased cohesin occupancy and H3K9 hypermethylation in the centromeric regions of cbf11 deletion cells is novel and interesting. However, the main deficiency of the manuscript is that this discovery is underdeveloped. For example, the evidence linking the mitotic defect phenotype to these two processes was not well supported. Moreover, there was no investigation in whether/how Cbf11 regulates cohesin occupancy or H3K9 methylation at the centromeres. Finally, the title and abstract provided an impression that lipid metabolism may influence cohesin occupancy and histone H3 hypermethylation at the centromeres, but this was not directly studied in the manuscript.

Centromeres are regions where sister chromatid cohesion is abolished last in mitosis. The observed higher levels of cohesin occupancy in the centromeric dh and dg repeats of cbf11 deletion cells could be the cause of chromosome missegregation, presumably because there is a delay or hinderance of cohesin removal from sister chromatids in mitosis. However, cohesin occupancy was carry out in asynchronous wild type and cbf11 deletion cultures, so it is unknown whether there is a delay of cohesion abolishment in mitosis. A cdc25-22 block and release experiment could better address this hypothesis. The observation that the spindle assembly checkpoint did not influence the mitotic catastrophe phenotype of cbf11 deletion cells suggests that the chromosome missegregation may not be mediated by defects in cohesin dynamics. How does Cbf11 influence cohesin dynamics in mitosis? Does Cbf11 regulate transcription of cohesin genes or indirectly through defects in the centromere or condensins?

There was no direct evidence that H3K9 hypermethylation at the centromeres contributes to the mitotic catastrophe phenotype of cbf11 deletion cells. It is also not clear whether Cbf11 directly or indirectly influences histone methylation at the centromeres of affect centromere function.

Based on a substantial number of protein-protein interactions of Cbf11 and gene products that affect chromatin function/silencing at the centromeres from the Pancaldi et al. 2012 study (e.g. HIR complex, Hrp1-Hrp3, Cnp1, Ino80 complex), I am surprised that these candidates were not mentioned in this study or investigated. Also, it would be more comprehensive to examine defects in transcriptional silencing in the centromeric regions using an ade6+ or ura4+/FOA marker system rather than measuring expression of per1+ and sdh1+.

Figure 1A shows that the "cut" and nuclear displacement phenotypes are independent. However, cut mutants can also generate a nuclear displacement phenotype [Samejima et al. (1993) J. Cell Sci. 105: 135-143]. Therefore, I am not sure whether the latter phenotype can be treated as entirely independent from "cut" mutants.

Significance

The discovery of increased cohesin occupancy and H3K9 hypermethylation in the centromeric regions of cbf11 deletion cells is novel and interesting. However, the main deficiency of the manuscript is that this discovery is underdeveloped.

The results of this manuscript would be of considerable interest in the area of cell cycle research, transcription and chromatin structure and function.

Reviewer 4

Evidence, reproducibility and clarity

Summary
In this paper Vishwanatha et al. analyze the mitotic phenotypes of cells lacking a regulator of lipid metabolism Cbf11. They propose that sister chromatid cohesion abnormalities and altered chromatin marks may contribute to the increased incidence of catastrophic mitosis. Additional experiments are required to improve the study and strengthen the authors' conclusions.

Major Comments
Both histone and alpha-tubulin tagging are known to aggravate mitotic errors in S. pombe. Before using these markers for live imaging, the authors should quantitate mitotic phenotypes in untagged cbf11Δ cells, as compared to the wild type. Using DAPI and Calcofluor staining (and ideally, also visualizing microtubules using anti- alpha-tubulin antibodies) the authors should measure the percentage of cells in mitosis and the percentage of cells that are going, or just went, through catastrophic mitosis, in asynchronous early-mid-exponential cell populations.

In analyzing the dynamic of nuclear division, the authors claim that the interval between spindle formation and anaphase onset is "longer" and "more variable" in cbf11Δ cells compared to WT cells. The authors should provide proper statistical analysis of both differences to show that these differences are significant. The same goes for the authors' claim that mitotic duration is "more variable" in cbf11Δ cells compared to WT cells. As mentioned above, alternative estimates of possible perturbations of mitotic dynamics could be obtained by measuring the percentage of cells in different mitotic phases in asynchronous untagged cell populations, in order to avoid possible artifacts given by tagging histones and alpha-tubulin.

The fact that inactivation of SAC does not change the incidence of catastrophic mitoses shows that SAC is not involved and that there are likely no problems with kinetochore- microtubule attachments. Therefore, the authors' statement "These results suggest that SAC activity only plays a minor role (if any) in the mitotic defects observed in cbf11Δ cells" should be changed. Also, the authors' statement in the conclusion that "This indicates that proper microtubule attachment to kinetochores might be compromised and takes longer to achieve in cbf11Δ cells, possibly triggering the SAC" should be changed accordingly or further proof should be provided.

As pointed out by the authors, cohesion occupancy is affected by the cell cycle phases duration. Therefore, the authors should correct their data (Fig.3C) for the different duration of mitosis or measure cohesion occupancy in mitotically synchronized populations. If this is not possible, I

suggest removing this piece of data altogether.

In Fig. 3A it is not clear what the authors mean by "morphological" differences between WT and cbf11Δ cells or between cbf11Δ cells and cbf11Δwpl1Δ cells. The authors should provide clearer images and indicate for each image which cells show morphological defects as an example.

In Fig. 3A many cells in single or double cbf11Δ mutants show increased size typical of diploid cells. The authors should perform flow cytometry to test for possible diploidization in their mutants, as that would clearly affect any conclusions on mitotic defects rescue or enhancement.

As correctly pointed out by the authors, it is not clear if the increase in mitotic defects in cbf11Δ cells is entirely due to the perturbed lipid metabolism or to other factors being affected by Cbf11. A possible approach to prove this point, as suggested by the authors too, would be to test if the mitotic defects identified in cbf11Δ are common to other mutants of lipid metabolism that also show an increase in catastrophic mitotic events. Also, the authors' statement in the conclusion: "we have demonstrated several novel factors, not directly related to lipid metabolism, that affect mitotic fidelity in cells with perturbed lipid homeostasis" should be modified as it was not proven that these effects are not due to altered lipid metabolism.

Minor comments

The initial distinction (Fig. 1A) between "cut" and "nuclear displacement" phenotypes is somewhat confusing, especially since the authors are not investigating the different outcomes of a catastrophic mitosis. The two outcomes should be grouped together under the definition of "catastrophic mitosis" as it is done in the rest of the paper.

I do not think I understand the statement that "SAC abolition might actually suppress the mitotic defects of the cbf11Δ cells". The lack of SAC might aggravate defects in kinetochore- microtubule attachment or other aspects of spindle assembly. If the authors know of specific examples where the deletion of mad2 or the genes encoding other SAC components rescued the mitotic defects, they should cite those papers. Either way, this point needs clarification.

Brightfield images in Fig. 1 would be clearer without the overlap of the fluorescence channels. The authors could also change the contrast of the images to highlight the septum.

The length of spindle (shown in Fig. S1) is a more informative measurement for mitotic dynamics and should be used instead of the "nuclear distance" presented in Fig. 2.

Generally, the authors could improve the data visualization by including in all the plots the single data points distribution along with the mean/median and error bars like it was done in Fig.2 C,D,E.

Significance

The paper expands the knowledge on Cbf11, a still poorly characterized regulator of lipid metabolism. The idea that in addition to nuclear membrane limitation, perturbations of lipid metabolism might cause mitotic chromosome dynamics defects (for instance, through changing the protein acetylation levels), is interesting, but the authors should strengthen their conclusions by performing controls and further experiments.

Author response to reviewers' comments

We would like to thank all reviewers for their time and effort invested into reviewing our manuscript.

Please find our responses to your comments, criticisms and suggestions below in blue.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

Summary:

The manuscript by Vishwanatha et al. presents findings on the fission yeast transcription factor Cbf11, which is involved in regulating lipid synthesis. Changes in lipid metabolism often have detrimental effects on nuclear division (evidenced by the high percentage of cut phenotypes among strains with altered lipid content). Here the authors show that cbf11 deletion strains produce additional phenotypes such as changes to cohesion dynamics and altered chromatin modification within centromeric regions, in turn perhaps affecting microtubule attachment and proper chromosome distributions. This hypothesis is supported by the authors' finding of epistatic effects between cbf11 and cohesin loading and unloading.

Major comments:
While the evidence presented supports the hypothesis of altered cohesin loading as a major driver of observed mitotic defects, changes in the NE surface area are likely to also contribute to the phenotypes even in pre-anaphase stages.

- This is an interesting notion. We are only aware of NE overproduction and nuclear “flares” observed upon the Lipin phosphatase dysregulation (PMID 23873576).
- However, in our case we rather expect NE membrane shortage, not overproduction. Accordingly, we do see that the nuclear cross section area (thus likely also NE surface area) is smaller in cbf11KO compared to WT (see boxplots below). Is this what you are referring to? We are not sure how this would affect the pre-anaphase stages of mitosis.

NOTE: We have removed unpublished data that had been provided for the referees in confidence.

Did the authors test any double deletions with regulators involved in decreasing lipid content (e.g. spo7, nem1, ned1) to counteract the role of Cbf11? This could be useful in assessing the relative contribution of cohesion dynamics and histone modifications.

- We previously published (PMID: 27687771) that cut6/ACC overexpression can indeed partially suppress the cut phenotype in the cbf11KO background. So lipid metabolism does play a role and does contribute to mitotic fidelity. In the current manuscript, we are showing that other factors contribute as well and that defects arise already prior to anaphase, which is not consistent with the simple notion of shortage of membrane building blocks during anaphase. We appreciate your suggestion on testing the relative contributions of these various factors to mitotic fidelity, but we have not tested any of the suggested double mutants.

A possible role of physical constraints dictated by the NE was already mentioned by the authors in the context of spindle bending and decreased elongation rates and some preliminary experimental data on this would be appreciated. Generation of strains, acquisition of some timelapses, and quantification of spindle elongation rate/buckling frequency should be feasible in a reasonable time frame.

- Assaying spindle parameters in Lipin-related mutants would indeed be interesting, but again, these are anaphase phenotypes. We are not sure how this is relevant for the pre-anaphase findings we report? Also, we unfortunately no longer have the personnel and capacity to carry out the suggested experiments.

The authors report mRNA levels of the centromere flanking genes per1 and sdh1 to be increased by 1.5x and decreased by 2x in comparison to WT. Could the authors elaborate on whether this is an expected trend? Kaufmann et al., 2010 reported low transcription of per1 when the surrounding regions are predominantly acetylated. Fig. 4A suggests a slight increase of H3K9ac at per1 and a decrease of transcription would be conceivable.

- We do not have any particular expectations regarding the expression levels of per1 and sdh1 in our system. We simply note that their expression changes in cbf11KO (in different directions) and this is accompanied by changes in H3K9 acetylation patterns.
- The increased histone acetylation at the per1 locus that you mention (Kaufmann et al., 2010) was only shown for H4K12ac, while we measured H3K9ac (these marks are deposited by different enzymes). The authors actually report that “The levels of histone H3 at per1 did not change significantly between the two growth conditions and strains”, so we do not think that paper is relevant for our study.

Fig. 3B indicates a catastrophic mitosis percentage of roughly 9.5% in cbf11Δ while in Fig. 1C 4% of all cells, or ˜31% of all mitotic events, is noted as abnormal. Could the authors clarify this discrepancy? Since Fig. 1 utilises time course data of 333 cells (please specify the number of analysed cells also in the legend), would the authors expect this data to be more trustworthy when compared to images of fixed cells? What were the criteria to assign divisions as catastrophic in fixed cells and which features were utilised to identify the 400 cells as mitotic?

- We typically do see higher proportions of cut cells in fixed samples than in live-cell imaging. We believe this has to do with the different fluorescence readouts for live vs fixed cells. We have added the following explanations to the methods:

  “Please note that the observed frequencies of mitotic defects are not directly comparable between live and fixed cells. Following catastrophic mitosis, the dead cells rapidly lose histone-GFP fluorescence (imaging of live cells), but their DNA can still be visualized with DAPI for a much longer period (imaging of fixed cells), resulting in higher apparent defect frequencies in fixed cells.”

- Importantly, we always compared cbf11KO to WT grown and processed under the same conditions, and that is how we determined the significance of any defects.
- Mitotic defects were classified based on nuclear morphology both in live cells (histone signal) and in fixed cells (DAPI): Cells having the cut phenotype, or mis-segregated nucleus = 2 nuclei of different sizes, or septated cells with only one daughter cell having a nucleus, respectively.
- We have analyzed images of at least 400 cells *in total* from asynchronous populations (interphase + mitotic >= 400). We have modified the figure legend to make this fact more clear. In our experience, this is the standard way of reporting the frequency of mitotic defects in asynchronous yeast cell populations.
- We have specified the number of cells analyzed in Fig. 1C.

Minor comments:
Previous literature is, to the best of our knowledge, sufficiently referenced. The text is largely clear (some exceptions within the methods section will be elaborated on below). The figures, however, would benefit from graph titles and some minor formatting changes.

- Figures:
o Fig. 1: Specify the number of cells analysed in C within the legend as well. For B, please use colourblind-friendly schemes - especially since images are shown as merges only. The example of the "cut" phenotype appears small and crowded by surrounding cells. Especially the latter might affect mitotic fidelity. Under the assumption that this did not affect quantifications (WT seem fine) a less crowded cell would present a nicer example.

- We have changed Fig. 1 as requested.

o Fig. 3: Images shown in A add little benefit in their current form. What is the takeaway for the reader?

- We hope that the reader gets concrete information on cellular and nuclear morphology of the investigated strains, which would be otherwise difficult to reproduce by textual description.

Indicating that images represent DAPI staining and pointing out cells of interest with arrows/symbols would be helpful.

- Done.

The example shown for cbf11 appears to be dimmer in comparison and cell morphology is hard to interpret.

- The cbf11KO cells stain fainter with DAPI than cells of other strains. We do not know why. To increase the clarity of the image, we have now adjusted the brightness and contrast of the cbf11KO panel (and indicated this adjustment in the figure legend).

C feels misplaced in this figure and a title could improve readability.

- We have added a title and moved the panel to Fig. 4 (4D).

o Fig. 4: Graph titles needed, figure might work better in portrait

- We have added the required graph titles.
- We have recreated all ChIP-seq related figures to incorporate new data and to (hopefully) better highlight the differences between genotypes.

- Text:
o Mention median duration of mitosis in cbf11Δ (Fig. 2E) in text since WT is already noted;

- Done.

o Discussion, third paragraph: "TBZ [REF] and are prone to chromosome loss [...]". I assume this referred to minichromosome loss or have changes in ploidy/chromosome segregation been quantified?

- Changes in ploidy were indeed not quantified. We have changed the wording to "**mini**chromosome loss". But please note that the Ch16 minichromosome is derived from regular Chromosome III and is a real chromosome, albeit a small one.

o Methods, Microscopy and image analysis:
How were fixed cells imaged (glass bottom dishes, plated on lectin, mounted on slides)?
Specify the CellR as widefield and provide details of the objective used (immersion and NA)

- We have added the following information to the relevant Methods section:

  "Cells were applied on glass slides coated with soybean lectin, covered with a glass cover slip, and imaged using the 60X objective of the Olympus CellR widefield microscope with oil immersion (NA 1.4)"

Elaborate on "manual evaluation of microscopic images"

- We have extended the description of cell scoring:
  "The frequency of catastrophic mitosis occurrence was determined by manual evaluation of microscopic images using the counter function of ImageJ software, version 1.52p (Schneider et al., 2012). At least 400 cells from the asynchronous populations were analyzed per sample and mitotic defects were scored based on nuclear morphology and septum presence/position. "

For live cell microscopy, what was the estimated final density of cells within the 5 µl resuspension?
- Our estimate is 4-8 x 10^6 cells in 5 ul. We have added this information into the Methods.

What is meant by measuring the maximum section of plotted profiles? Is this the maximum distance of Hht1 signals within the entire time-lapse?

- We have changed the description:

> "The nuclear distance was measured by using Hht2–GFP signals and converting the green channel images to binary, measuring the maximum distance between the Hht2-GFP signals using plot profile function in imageJ."

Was spindle length quantified the same way?

- We have added the description:

> "Spindle length was quantified by drawing a line along the length of the spindle (using mCherry-Atb2 signals) at each timepoint and measuring the length of the line using imageJ."

Methods, ChIP-qPCR:
It is not clear which strains were used, this can only be guessed by the use of a GFP antibody suggesting GFP tagged chromatin to be precipitated. For people with expertise outside of ChIP assays, this should be specified

- We have listed the used strains in the ChIP-qPCR methods section.

Reviewer #1 (Significance (Required)):

Describe the nature and significance of the advance (e.g. conceptual, technical, clinical) for the field.

This manuscript presents a novel role for a transcription factor, one typically implicated in lipid metabolism, in chromatin modification and cohesin dynamics, with the possibility of this representing a more conserved process across ascomycetes. The mechanism of cbf11 regulation remains to be determined.

Place the work in the context of the existing literature (provide references, where appropriate).

This work helps link two bodies of work related to cell division that are usually considered in isolation, the regulation of lipid dynamics and the control of chromatin dynamics and cohesion. Some comparisons to phenotypes in closely related species would have helped provide a broader context (such as Yam et al., 2011, where the spindle morphologies in S. japonicus and response to cerulenin treatment might be of relevance to the work presented here).

- We now briefly discuss the semi-open mitosis of Sch. japonicus and the Yam et al. 2011 paper at the beginning of the Discussion.

State what audience might be interested in and influenced by the reported findings. Molecular and cellular biologists with interests in nuclear remodelling, lipid metabolism, kinetochore assembly.

Define your field of expertise with a few keywords to help the authors contextualize your point of view. Indicate if there are any parts of the paper that you do not have sufficient expertise to evaluate.

Fission yeast biology, nuclear remodelling, microscopy. We are not qualified to make in-depth comments on the soundness of ChIP-Seq and ChIP-qPCR experiments.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

This manuscript describes detailed mechanisms by which the cbf11 deletion showed the phenotype. They found that the cbf11 deletion altered pericentromeric chromatin states such as the level of cohesin and hypermethylation.

In general, their results are interesting and provide important insights into the relationship between lipid metabolism and chromosome segregation. The presented data are valuable for the community, but the authors should carefully re-assess their data.

Major comments:
1. Statistical analyses in some of the Fig.3B, 3C, 4B and S2 seem to be somewhat weird because p-values are too small for such a small number of experiments (three independent experiments) with large standard deviations. Please show all the data points in Fig. 2C-E, and provide raw values as a supplementary table for assessment of the data.

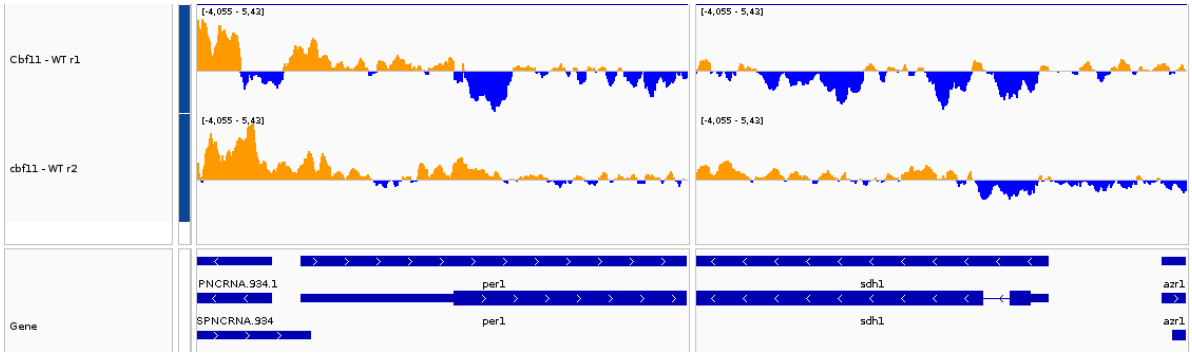
- We now show individual data points for all barplots and boxplots and provide all source numerical data as supplementary tables. The details of the used statistical tests are given in the respective figure legends.

2. Pages 5-6: As for Fig. 4, the data is difficult to interpret because the trends of the ChIP-seq pattern of H3K9me2 between replicates look different: replicate 2 shows an increase of H3K9me2 signal, while replicate 1 shows almost no difference or weak if any. In such a case, the authors should repeat ChIP-seq one more time and confirm hypermethylation at these regions or confirm it by ChIP-qPCR.

- We do not agree with this statement. It is true that the exact histone modification patterns are not identical between the two replicates, but this is likely due to the differences in chromatin extract preparation in replicate 1 vs replicate 2 (see Methods). Importantly, both replicates show pronounced differences in H3K9me2 patterns between WT and cbf11KO. We have changed the visualization style to better highlight the differences between WT and mutant (Fig. 4A, Fig. S2B, S3)).
- Also, we have added one more biological replicate for the H3K9me2 ChIP-seq (Fig. S3) and performed the H3K9me2 ChIP-seq also in the Pcut6MUT strain with ~50% decreased expression of the cut6 gene (Cut6/ACC is the rate-limiting enzyme of fatty acid synthesis; cut6 is target of Cbf11) as 3 biological replicates (Fig. 4A and Fig. S3). Importantly, all replicates of both mutant strains show hypermethylated regions in the centromeres compared to WT.

Assuming that the pericentromeric regions are hypermethylated by cbf11 deletion, it is still unclear why the transcription from only dh, but not dg, regions increased although their ChIP-seq data indicated both dh/dg regions were hypermethylated. A similar question arises to the expression of per1 and sdh1. Both K9Ac and K9me2 modifications seem to unchange at both per1 and sdh1 loci, whereas the expression levels of these loci changed in the opposite direction. These results suggest that the transcription levels of the centromeric region are independent of their histone modification states.

- We do not know why dh expression differs from dg. But note that these are multi-copy repeats and it is very difficult to study individual copies separately. Our expression data, and partly also the ChIP-seq data represent “average” values across all the dh and dg copies present in the genome.
- Importantly, Figure 4A (and Fig. S2B, S3) show a large piece of the fission yeast chromosome (~57 kbp) and this scale does not allow making informed judgements about the state of histone modifications at a particular promoter locus.
- When we zoom in, we do see increased and decreased H3K9ac around the TSS of per1 and sdh1, respectively (2 replicates shown).



3. A key question of this study is to understand the relationship between lipid metabolism and chromosome structures. However, the results presented are not enough to address this question. I request to distinguish whether the defects on pericentromeric regions are mediated by lipid metabolism or direct effect by cbf11 deletion. Cbf11 is a transcription factor and can directly bind to DNA, thereby there is a possibility that Cbf11 directly modulates the pericentromeric chromatin state without regulating lipid metabolism. This question can probably be addressed. As the authors have shown in their previous study (Prevorovsky et al., 2016), overexpression of cut6, which encodes acetyl coenzyme A carboxylase and is a target of cbf11, can bypass nuclear defects. If the overexpression of cut6 restores alteration on pericentromeric regions such as cohesin enrichment and hypermethylation, it suggests the defects are a secondary effect of the decrease of phospholipid biosynthesis.

- We agree that any rescue effects can be direct or indirect. And distinguishing between these two alternatives is unfortunately not straightforward.
- Our Cbf11 ChIP-seq data do not show Cbf11 binding to centromeres (PMID 19101542), suggesting that any impact of Cbf11 on centromeric chromatin is most likely indirect and mediated by some other, downstream, players.
- Instead of assaying cut6OE, we now show data that decreased cut6/ACC (a target of Cbf11) expression also leads to changes in histone methylation, similar to cbf11KO (Fig. 4A, Fig. S3). This suggests that lipid metabolism indeed can affect chromatin state (and the chromatin defects in cbf11KO are likely also lipid-related).
- We have recently shown (Princová et al., 2023, PMID: 36626368) that decreased fatty acid synthesis leads to changes in acetylation and expression of specific stress-response genes in S. pombe, and the whole process involves the histone acetyltransferases Gcn5 and Mst1. Therefore, instead of implicating membrane phospholipids, we rather suggest that lipid metabolism can affect chromatin acetylation/methylation and structure via HATs, potentially through acetyl-CoA, the common substrate of both FA synthesis and HATs. We now mention the Princová et al., 2023 paper in the Discussion section.

Minor comments:
4. Figure 3C: The legend says, "Values represent means + SD from 3 independent experiments". It meant "means {plus minus} SD"?

- Corrected. Thank you for spotting this.

5. The relationship between phospholipid synthesis and mitotic fidelity is now discussed in the bioRxiv paper (https://doi.org/10.1101/2022.06.01.494365). It would be nice to discuss this paper.

- Thank you for pointing out this reference. We now briefly mention this paper as a note that dysregulation of membrane phospholipid synthesis leads to mitotic phenotypes similar to cbf11KO.

Reviewer #2 (Significance (Required)):

Faithful chromosome segregation into daughter cells is crucial for cell proliferation. The authors previously reported that the deletion of cbf11, a transcription factor that regulates lipid metabolism genes, causes "cut (cell untimely torn)" phenotype (Prevorovsky et al., 2015;

Prevorovsky et al., 2016). In this report, they examined detailed mechanisms by which the cbf11 deletion showed the phenotype, and found that the cbf11 deletion altered pericentromeric chromatin states such as the level of cohesin and hypermethylation. In general, their results are interesting and provide important insights into the relationship between lipid metabolism and chromosome segregation. The presented data are valuable for the community of basic science in the fields of chromosome biology and cell biology.

We are cell biologists working on chromosomes and the cell nucleus.

Reviewer #3 (Evidence, reproducibility and clarity (Required)):

The Vishwanatha et al. manuscript examined the nature of the mitotic defect in cbf11 deletion cells. cbf11+ encodes a CSL transcription factor that regulates lipid metabolism genes in S. pombe. Loss of cbf11+ was previously shown to have a "cut" phenotype presumably due in part to aberrant regulation of its target gene cut6+ which encodes-acetyl CoA/biotin carboxylase involved in fatty acid biosynthesis (Zach et al. 2018). The authors hypothesized that the mitotic defect exhibited as chromosome missegregation in cbf11 deletion cells may be caused by alterations in cohesin occupancy and H3K9 methylation in centromeres. Cohesin occupancy was slightly higher in centromeric dh and dg repeats in the cbf11 mutant and loss of the cohesin-loader gene wpl1+ appeared to suppress the mitotic defect. The authors also showed by ChIP-Seq that H3K9 methylation was higher in the centromeric regions, as well as increased minichromosomal loss in the cbf11 mutant.

The discovery of increased cohesin occupancy and H3K9 hypermethylation in the centromeric regions of cbf11 deletion cells is novel and interesting. However, the main deficiency of the manuscript is that this discovery is underdeveloped. For example, the evidence linking the mitotic defect phenotype to these two processes was not well supported.

- We believe that the links have already been well established in the literature. The integrity of centromeric heterochromatin (H3K9me2) is known to be required for mitotic fidelity (eg. Clr4/HMT and Clr6/HDAC mutants with H3K9me2 deficiency have high minichromosome loss and/or show lagging chromosomes during mitosis - PMID: 19556509, PMID: 8937982, PMID: 9755190). Moreover, we stress the known interconnections and provide relevant citations in the Discussion:

  “It is also important to note that heterochromatin, kinetochore function, cohesin occupancy, and gene expression are all interconnected and actually interdependent (Bernard et al., 2001; Folco et al., 2019, 5; Grewal and Jia, 2007; Gullerova and Proudfoot, 2008; Nonaka et al., 2002; Volpe et al., 2002)”

- We show in the manuscript altered cohesin occupancy in cbf11KO and show that mutations in cohesin loading factors do affect mitotic fidelity of cbf11KO. While we do agree that this connection can be developed further, we believe this is beyond the scope of our current project.

Moreover, there was no investigation in whether/how Cbf11 regulates cohesin occupancy or H3K9 methylation at the centromeres.

- This is true. But again, we believe this is beyond the scope of our current project.

Finally, the title and abstract provided an impression that lipid metabolism may influence cohesin occupancy and histone H3 hypermethylation at the centromeres, but this was not directly studied in the manuscript.

- We now provide H3K9me2 ChIP-seq data on the Pcut6MUT mutant deficient in fatty acid synthesis to show that lipid metabolism indeed can affect histone methylation at the centromeres (Fig. 4A, Fig. S3).

Centromeres are regions where sister chromatid cohesion is abolished last in mitosis. The observed higher levels of cohesin occupancy in the centromeric dh and dg repeats of cbf11 deletion cells could be the cause of chromosome missegregation, presumably because there is a delay or hinderance of cohesin removal from sister chromatids in mitosis. However, cohesin occupancy was carry out in asynchronous wild type and cbf11 deletion cultures, so it is unknown whether there is a delay of cohesion abolishment in mitosis. A cdc25-22 block and release experiment could better address this hypothesis.

- We acknowledge these limitations of our findings regarding cohesin occupancy in the paper:

  " Notably, centromeres are the regions where sister chromatin cohesion is abolished last during mitosis (Peters et al., 2008). Since cbf11Δ cells show altered cell-cycle and pre-anaphase mitotic duration compared to WT (Fig. 2), the observed difference in cohesin occupancy might merely reflect these changes in the timing of cell cycle progression. Alternatively, altered cohesin dynamics could play a role in the cbf11Δ mitotic defects."

- We agree the issue could be addressed better using synchronous cell populations. However, the cdc25 or cdc10 block-release does not work well in cbf11KO (PMID: 27687771), and we currently do not have the capacity to perform less disruptive forms of cell cycle synchronization.

The observation that the spindle assembly checkpoint did not influence the mitotic catastrophe phenotype of cbf11 deletion cells suggests that the chromosome missegregation may not be mediated by defects in cohesin dynamics. How does Cbf11 influence cohesin dynamics in mitosis?

- There are clearly multiple contributors to the mitotic defects observed in the cbf11KO strain and we state this explicitly throughout the manuscript.
- We agree that it would be interesting in future to know more details about the link between Cbf11 and cohesin, but this is beyond the scope of our current project.

Does Cbf11 regulate transcription of cohesin genes or indirectly through defects in the centromere or condensins?

- Expression levels of cohesin and condensin genes are not affected by deletion of cbf11 (PMID: 26366556). We now mention these findings in the Results section.

There was no direct evidence that H3K9 hypermethylation at the centromeres contributes to the mitotic catastrophe phenotype of cbf11 deletion cells.

- This is true. However, the importance of H3K9me2 for mitotic fidelity has already been established in the literature (as we mention above).

It is also not clear whether Cbf11 directly or indirectly influences histone methylation at the centromeres of affect centromere function.

- When the Cbf11 protein is missing, centromeric histone methylation is different from normal (WT), and centromere function is not normal either - dh repeats are less expressed, minichromosome derived from ChrIII (so has a normal centromere) is 9x more frequently lost. So Cbf11 does affect these processes. The question remains, whether Cbf11 does this directly or indirectly. We favor the indirect route, as we have recently shown that H3K9 acetylation or methylation can be affected by shifting the balance between fatty acid synthesis (which is regulated by Cbf11) and histone acetyltransferase activity. We now mention these findings in the Discussion (Princová et al., 2023).

Based on a substantial number of protein-protein interactions of Cbf11 and gene products that affect chromatin function/silencing at the centromeres from the Pancaldi et al. 2012 study (e.g. HIR complex, Hrp1-Hrp3, Cnp1, Ino80 complex), I am surprised that these candidates were not mentioned in this study or investigated.

- Unfortunately, no DNase treatment was used during the affinity purification of Cbf11 in the study you mention. Therefore, the list of potential interactors is likely contaminated by irrelevant, DNA-mediated interactions with proteins sitting at nearby loci. This is why we have not pursued these candidates.

Also, it would be more comprehensive to examine defects in transcriptional silencing in the centromeric regions using an ade6+ or ura4+/FOA marker system rather than measuring expression of per1+ and sdh1+.

- We agree. We actually tried the ura4/FOA reporter system, but had problems constructing the reporter strains in the cbf11KO background. The resulting clones showed variable levels of FOA sensitivity (see figure of clones OC5-9 below), so we could not get a conclusive answer from this experiment and resorted to measuring the expression of pericentromeric genes.

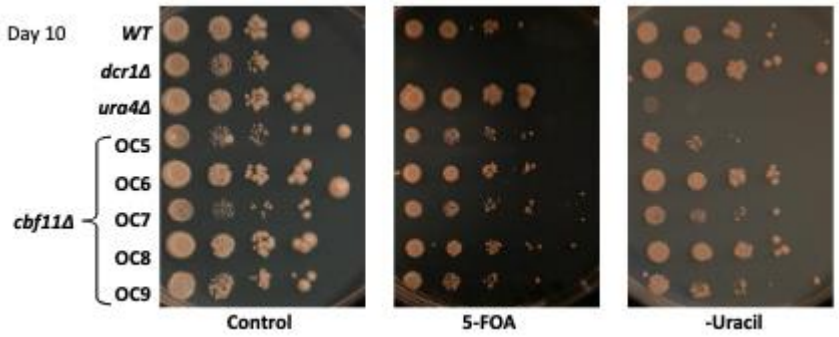


Figure 1A shows that the "cut" and nuclear displacement phenotypes are independent. However, cut mutants can also generate a nuclear displacement phenotype [Samejima et al. (1993) J. Cell Sci. 105: 135-143]. Therefore, I am not sure whether the latter phenotype can be treated as entirely independent from "cut" mutants.

- We have made clarifications to Fig. 1A accordingly.

Reviewer #3 (Significance (Required)):

The discovery of increased cohesin occupancy and H3K9 hypermethylation in the centromeric regions of cbf11 deletion cells is novel and interesting. However, the main deficiency of the manuscript is that this discovery is underdeveloped.

The results of this manuscript would be of considerable interest in the area of cell cycle research, transcription and chromatin structure and function.

Reviewer #4 (Evidence, reproducibility and clarity (Required)):

Summary

In this paper Vishwanatha et al. analyze the mitotic phenotypes of cells lacking a regulator of lipid metabolism Cbf11. They propose that sister chromatid cohesion abnormalities and altered chromatin marks may contribute to the increased incidence of catastrophic mitosis. Additional experiments are required to improve the study and strengthen the authors' conclusions.

Major Comments

Both histone and alpha-tubulin tagging are known to aggravate mitotic errors in S. pombe. Before using these markers for live imaging, the authors should quantitate mitotic phenotypes in untagged cbf11Δ cells, as compared to the wild type. Using DAPI and Calcofluor staining (and ideally, also visualizing microtubules using anti- alpha-tubulin antibodies) the authors should measure the

percentage of cells in mitosis and the percentage of cells that are going, or just went, through catastrophic mitosis, in asynchronous early-mid-exponential cell populations.

- We agree that tagging can affect protein function in numerous ways.
- The tagged versions of tubulin (mCherry-Atb2) and H3 (Hht2-GFP) used in our paper have been obtained from Phong Tran's lab. These tagged alleles had been published (Nature Communications, PMID: 26031557) and used successfully to monitor mitotic defects including chromosome segregation errors and the cut phenotype.
- The analyses of mitotic and septation defects of asynchronous untagged cbf11KO cells that you suggest (except for the spindle visualization) were already done by us (PMID: 19101542, PMID: 26366556) and are in agreement with our present study. In brief, we showed that cbf11KO populations contain ~10-30% of cells with mitotic defects (eg. cut), depending on the cultivation conditions. They also show septation defects and altered cell morphology and shorter cell length.

In analyzing the dynamic of nuclear division, the authors claim that the interval between spindle formation and anaphase onset is "longer" and "more variable" in cbf11Δ cells compared to WT cells. The authors should provide proper statistical analysis of both differences to show that these differences are significant.

- We now show the required data and statistical testing as Fig. 2H.

The same goes for the authors' claim that mitotic duration is "more variable" in cbf11Δ cells compared to WT cells.

- The spread of values for both WT and cbf11KO is given in Fig. 2G.

As mentioned above, alternative estimates of possible perturbations of mitotic dynamics could be obtained by measuring the percentage of cells in different mitotic phases in asynchronous untagged cell populations, in order to avoid possible artifacts given by tagging histones and alpha-tubulin.

- As you mention above, to estimate their cell cycle stage, untagged cells would need to be fixed and stained to visualize the nucleus and septum. However, using fixed cbf11KO cells is not optimal for this purpose. cbf11KO have septation and cell separation defects (PMID: 19101542, PMID: 26366556). This results in increased numbers of cells having a (persistent) septum in the asynchronous population, which obscures any estimates of cell cycle stages, and this is why we observed live cells during a timecourse.

The fact that inactivation of SAC does not change the incidence of catastrophic mitoses shows that SAC is not involved and that there are likely no problems with kinetochore-microtubule attachments. Therefore, the authors' statement "These results suggest that SAC activity only plays a minor role (if any) in the mitotic defects observed in cbf11Δ cells" should be changed.

- We have changed the sentence to:

  "These results suggest that SAC activity only plays a minor role (if any) in the mitotic defects observed in cbf11Δ cells, or that the defects are not caused by problems with kinetochore-microtubule attachment."

Also, the authors' statement in the conclusion that "This indicates that proper microtubule attachment to kinetochores might be compromised and takes longer to achieve in cbf11Δ cells, possibly triggering the SAC" should be changed accordingly or further proof should be provided.

- This is probably a misunderstanding. We do not conclude that failed microtubule attachment to kinetochores is surely the cause of mitotic defects in cbf11KO. We merely describe our reasoning about structuring the project during its execution. We have rephrased the problematic sentence to improve clarity.
- We already state in the Discussion that the mitotic defects of cbf11KO may be caused by something completely different from microtubule attachment.

As pointed out by the authors, cohesion occupancy is affected by the cell cycle phases duration. Therefore, the authors should correct their data (Fig.3C) for the different duration of mitosis or measure cohesion occupancy in mitotically synchronized populations. If this is not possible, I suggest removing this piece of data altogether.

- We agree (and acknowledge in the paper) that the measurement of cohesin occupancy can be affected by duration of mitotic phases. However we do not see a straightforward way of normalizing for mitotic duration, as cohesin occupancy changes differentially at particular chromosomal loci.
- The suggested experiment of measuring cohesin occupancy in synchronized mitotic cells would likely help. However, as mentioned in our response to Reviewer 3 above, the cdc25 or cdc10 block-release does not work well in cbf11KO (PMID: 27687771), and the heat shock or drugs (eg. spindle poisons) would introduce confounding issues themselves. Unfortunately, we currently do not have the capacity to perform less disruptive forms of cell cycle synchronization.
- Since we show that mutations in cohesin loading factors can rescue mitotic fidelity of cbf11KO cells (Fig. 3B), we consider the data shown in Fig. 3C relevant. Therefore, we opt to keep Fig. 3C in the paper, and we do point out the potential limitations of these results in the Results section.

In Fig. 3A it is not clear what the authors mean by "morphological" differences between WT and cbf11Δ cells or between cbf11Δ cells and cbf11Δwpl1Δ cells. The authors should provide clearer images and indicate for each image which cells show morphological defects as an example.

- We now use arrows to highlight cells with nuclear defects in Fig. 3A.
- We now state examples of the cbf11KO-associated morphological defects in the text, together with a reference to the paper describing these defects in detail.

In Fig. 3A many cells in single or double cbf11Δ mutants show increased size typical of diploid cells. The authors should perform flow cytometry to test for possible diploidization in their mutants, as that would clearly affect any conclusions on mitotic defects rescue or enhancement.

- We previously published that cbf11KO cells show increased tendency for spontaneous diploidization (PMID: 19101542). When constructing cbf11KO strains, we always take care (including flow cytometry tests of DNA content) to exclude purely diploid clones, but the process of spurious diploidization is continuous and there are always diploid cells present in the cbf11KO culture.
- We mention diploidization as a possible mitotic outcome in cbf11KO cells in the first section of the Results.

As correctly pointed out by the authors, it is not clear if the increase in mitotic defects in cbf11Δ cells is entirely due to the perturbed lipid metabolism or to other factors being affected by Cbf11. A possible approach to prove this point, as suggested by the authors too, would be to test if the mitotic defects identified in cbf11Δ are common to other mutants of lipid metabolism that also show an increase in catastrophic mitotic events.

- We now show ChIP-seq data showing that centromeric H3K9 shows aberrant methylation patterns also in a hypomorphic cut6/ACC mutant (Pcut6MUT) (Fig. 4A, Fig. S3).
- We previously showed that the Pcut6MUT mutation predisposes fission yeast cells to catastrophic mitosis, and the defects manifest when Cut6 function is further weakened by limiting the supply of biotin (cofactor of Cut6) (PMID: 27687771).

Also, the authors' statement in the conclusion: "we have demonstrated several novel factors, not directly related to lipid metabolism, that affect mitotic fidelity in cells with perturbed lipid homeostasis" should be modified as it was not proven that these effects are not due to altered lipid metabolism.

- We agree that “it was not proven that these effects are not due to altered lipid metabolism”. However, the emphasis here is on the word “directly”. H3K9me2 and cohesin

dynamics are not directly related to the metabolism of lipids. We have changed the phrasing to improve clarity.

Minor comments

The initial distinction (Fig. 1A) between "cut" and "nuclear displacement" phenotypes is somewhat confusing, especially since the authors are not investigating the different outcomes of a catastrophic mitosis. The two outcomes should be grouped together under the definition of "catastrophic mitosis" as it is done in the rest of the paper.

- We have changed Fig. 1A accordingly.

I do not think I understand the statement that "SAC abolition might actually suppress the mitotic defects of the cbf11Δ cells". The lack of SAC might aggravate defects in kinetochore-microtubule attachment or other aspects of spindle assembly. If the authors know of specific examples where the deletion of mad2 or the genes encoding other SAC components rescued the mitotic defects, they should cite those papers. Either way, this point needs clarification.

- We already provide an example in the Discussion:

  "Intriguingly, SAC inactivation has been shown to suppress the temperature sensitivity of the cut9-665 APC/C mutant, which is also prone to catastrophic mitosis (Elmore et al., 2014)"

- We have now included this reference and explanation also at the point in the text that you are referring to.

Brightfield images in Fig. 1 would be clearer without the overlap of the fluorescence channels. The authors could also change the contrast of the images to highlight the septum.

- We have changed Fig. 1B as requested.

The length of spindle (shown in Fig. S1) is a more informative measurement for mitotic dynamics and should be used instead of the "nuclear distance" presented in Fig. 2.

- This might be true for a successful mitosis. But in case of defects (such as spindle detachment from the chromosomes, regressive merger of the daughter nuclei), these parameters become partially uncoupled and both are informative. We have therefore included the data from Fig. S1 in new Fig. 2C-D.

Generally, the authors could improve the data visualization by including in all the plots the single data points distribution along with the mean/median and error bars like it was done in Fig.2 C,D,E.

- Done.

Reviewer #4 (Significance (Required)):

The paper expands the knowledge on Cbf11, a still poorly characterized regulator of lipid metabolism. The idea that in addition to nuclear membrane limitation, perturbations of lipid metabolism might cause mitotic chromosome dynamics defects (for instance, through changing the protein acetylation levels), is interesting, but the authors should strengthen their conclusions by performing controls and further experiments.

Original submission

First decision letter

MS ID#: JOCES/2023/261265

MS TITLE: Altered cohesin dynamics and histone H3K9 modifications contribute to mitotic defects in the cbf11Δ" lipid metabolism mutant

AUTHORS: Akshay Vishwanatha, Jarmila Princova, Patrik Hohos, Robert Zach, and Martin Prevorovsky

ARTICLE TYPE: Research Article

Thank you for sending your manuscript to Journal of Cell Science through Review Commons.

As you will see from their reports, the reviewers' recommendations are mixed. While referee #1 supports publication, referee #2 considers that you did not adequately revised your manuscript. After carefully reading your revised study and all initial review commons reviews, I am happy to tell you that your manuscript is acceptable for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

I reviewed this paper for Review Commons. An updated version of my summary:

The manuscript by Vishwanatha et al. presents findings on the fission yeast transcription factor Cbf11, which is involved in regulating lipid synthesis.
Changes in lipid metabolism often have detrimental effects on nuclear division (evidenced by the high percentage of cut phenotypes among strains with altered lipid content). Here the authors show that cbf11 deletion strains produce additional phenotypes such as changes to cohesion dynamics and altered chromatin modification within centromeric regions, in turn perhaps affecting microtubule attachment and proper chromosome distributions. This hypothesis is supported by the authors' finding of epistatic effects between cbf11 and cohesin loading and unloading.

This manuscript presents a novel role for a transcription factor, one typically implicated in lipid metabolism, in chromatin modification and cohesin dynamics with the possibility of this representing a more conserved process across ascomycetes. The mechanism of cbf11 regulation remains to be determined. This work helps link two bodies of work related to cell division that are usually considered in isolation, the regulation of lipid dynamics and the control of chromatin dynamics and cohesion. Work in closely related species provides a broader context (e.g. Yam et al., 2011, where the spindle morphologies in S. japonicus and response to cerulenin treatment are of relevance to the work presented here).

Comments for the author

The authors have satisfactorily addressed feedback and concerns I raised during review. The authors declined to carry out a few suggested experiments to provide additional context but I would not consider these essential for publication.

Reviewer 2

Advance summary and potential significance to field

I cannot confirm that the manuscript has been adequately revised.

Comments for the author

The point-by-point responses do not clearly explain how revisions have been made.