CENP-T regulates both the G2/M transition and anaphase entry by acting through CDH1 in meiotic oocytes

Yue Wang, Jian Li, Feng Dong, Wei Yue, Yin-Chun Ouyang, Zhen-Bo Wang, Yi Hou, Heide Schatten and Qing-Yuan Sun
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Original submission

First decision letter

MS ID#: JOCES/2019/238105

MS TITLE: CenpT, a kinetochore protein, regulates both G2/M transition and anaphase entry by acting through Cdh1 in meiotic oocytes

AUTHORS: Yue Wang, Jian Li, Feng Dong, Wei Yue, Yin-Chun Ouyang, Zhen-Bo Wang, Yi Hou, Heide Schatten, and Qing-Yuan Sun
ARTICLE TYPE: Research Article

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

The authors examine the meiotic function of CenpT in mouse oocytes using an antisense knockdown approach. They discover, as far as I am aware, quite a novel ability of this protein to regulate the APC activator Cdh1/Fzr1. Because APC(FZR1) regulates the timing and ability of oocytes to complete meiosis I then knockdown or overexpression also affects these processes.
The novelty of the findings, and the connection between a kinetochore component and a major cell cycle regulator make this topic of interest and I am supportive of this work. However I make the following suggestions for improvement as I was disappointed in the standard of presentation, the analysis performed and the clarity of the text.

Comments for the author

Details are:

1. Introduction. Is a little long and rambling. It could be made shorter and only include information explaining role of APC-FZR1 in mouse oocytes, and the examination here of role of CENP-T in regulating its activity. Much of the extra information could be incorporated into the Discussion.
2. All figures. State how many repeats in the figure legend for each experiment
3. Blots. All of the western blots in the paper are highly cropped. This is ok for presentation style. I would suggest for complete disclosure best to have full blots given in SI.
4. Fig 2. Using SEM here is not really informative. I'd like to know the spread of the data. Suggest using box plots or using sd. Fig 2D is particularly uninformative as displayed, and suggest to add some annotation to it.
5. Line 128. "Thus, CenpT depletion causes prophase I arrest of oocytes by inhibiting MPF activity." Not strictly correct. More correctly: The arrest is associated with lack of MPF activity caused by loss of cyclin B1 and lack of CDK1 dephosphorylation.
6. "negative control MO"…. Detail needed in Materials and Methods. Currently no sequence is given.
7. Statistical analysis. Much of the data presented involves a comparison of just two groups (with or without MO for example). The Methods state ANOVA was used. However in these instances surely another test is being used? Also the posthoc test applied for ANOVA is a little unusual. Better to use Tukeys, which is generally regarded as being less liable to giving a Type 1 error.
8. "We also found that oocytes at the MII stage showed increased percentages of abnormal chromosome distribution in CenpT mRNA injected oocytes (Fig. 5D, E)". I find the terminology of abnormal chromosome distribution to be vague and the figures unhelpful in clarifying what is considered normal.
Authors could be more descriptive of what abnormalities are being seen and how they are objectively being scored.
9. Was there reference to the SI movies in the manuscript? In any case their inclusion is probably unneeded. They are currently poor resolution brightfield timelapses that are not so informative.
10. Discussion. I do not see need to reference figures again in the text. It almost reads as a repeat of the Results. Authors should move references to figures.
11. There are many small typos and badly phrased sentences in the submission. For example: Line 110 GV stage were collected form female mice at six weeks of age. Should be 'from'.

Reviewer 2

Advance summary and potential significance to field

In this study, the authors found a new role of kinetochore protein Cenp T in regulating G2/M transition of meiotic oocytes. The authors provided evidences proving that Cenp T could affect MPF activity through regulating cdh1 level in mouse oocytes. The finding is interesting and contributes to better understanding the meiosis of mammalian oocytes. However, before the manuscript could be accepted for possible publication, the authors needs to address the following concerns.

Comments for the author

1. No specific scientific question raised by the researchers has been shown in either Abstract or Introduction part.
2. The Discussion part looks more like a duplicate of the major results, rather than a well organized discussion of the major conclusion of the research.
Therefore, the authors needs to rewrite the part.
3. The illustrations in several figures are not accurate or lack of detailed information, which includes Fig.3 E, Fig.4 CD, Fig.5 A and Fig.6 DE. Please refine them.
4. The statistic significance indication are not unanimously marked as the rest figures, such as Fig.5 D, Fig. 6 ADE and Fig. 7E, which did not have lines to indicates the differences between the control and the experimental group.
5. The fonts of the words within the manuscript are not consistant.

Reviewer 3

Advance summary and potential significance to field

Kinetochore, a proteinaceous structure of the centromere, is known to be essential for supporting chromosomal segregation during both mitosis and meiosis. However, the exact function of the individual component of kinetochores in the control of cell division, particularly in mammalian oocytes, remains largely undefined. In this manuscript, Wang et al., demonstrated that CENPT regulates the resumption of the first meiosis (GVB) in mouse oocytes, which largely differs from the reported role of CENPT in mitosis. They found that knocking down of Cenpt using siRNAs causes the inhibition of oocyte spontaneous GVB, which is associated with the elevation of the levels of CDH1 but decrease in the levels of CCNB1 and the activity of MPF. Cenpt-siRNA caused GVB inhibition is rescued by knockdown of CDH1. They also found that over-expression of CENPT in mouse oocytes causes the premature inactivation of SAC, as well as acceleration of the completion of the first meiosis and misalignment of chromosomes in the resulted MII oocytes. These meiotic defects are associated with the reduced levels of CDH1 and the earlier degradation of CCNB1 in maturing oocytes. Based on these observations, they concluded that CENPT regulates oocyte meiotic maturation by acting through CDH1.

This is an interesting study that addressed a key scientific question in the oocyte biology field, and is hence of broad interest to the cell biology community. The results presented here are novel, and will certainly help to advance this field significantly.

Comments for the author

There are several points need to be addressed carefully.

Major concerns:

1) The major weakness of this study is the lack of mechanism that link CENPT and CDH1. How is the function of CENPT connected to CDH1? Is it a direct interaction between CENPT and CDH1?

2) The function of CENPT in the control of oocyte meiotic progression needs to be tested more vigorously by knockdown of CENPT in GVB oocytes. Acute deletion of CENPT in GVB oocytes by TrimAway method is highly recommended.

3) In the GV stage, knockdown of CENPT caused the upregulation of CDH1 that in turns reduced the levels of CCNB1 and MPF activity; while in GVB oocytes, over-
expression of CENPT lowered down the levels of CDH1, but CCNB1 was also prematurely degraded. How did this happen? Does this mean that there is different mechanism(s) operating at GV stage and MI-anaphase I transition? Is CDC20 prematurely activated? Is securin also prematurely degraded?

4) A more focused and succinct Discussion is required. Please get rid of the redundant repeat of the results in the Discussion.

5) A diagram is suggested to demonstrate the major findings of this study.

6) The upregulation of CENPT at GVB stage is very intriguing. Is it due to the activation of translation? What is the potential function of this dramatic increase? This needs to be discussed. Also, should this increase be reflected by the IF staining?

Some minor issues:
1) Standard nomenclature of the mouse genes, mRNAs, and proteins are highly recommended.

2)The format of the reference needs to be carefully checked.

3)Page 2, line 56-57, this is an awkward sentence.

4) Page 3, line 68-70, this sentence needs to be modified. Line 77, What is "T-terminal"? Line 77-84, this part need to be modified.

5) Page 4, line 88, "the meiotic cell cycle" is highly recommended to be changed into "meiotic progression". The same as to line 98. Line 91, Is it a real "continuous degradation"?

6) Page 4, line 114, How about extend the culture up to 20h?

7) Page 5, line 130, "low levels of cyclin B1"≠ "cyclin B1 malfunction".

8) Page 6, line 147, "collecting them" should be "collected". Line 151, "translation" should be "transition". Line 150-170, this part needs to be modified, paying attention to use passive tense, and the results need to stated precisely.

9) Page 7, line 174, what does "realized" mean here?

10) Page 8, line 213-215, this sentence needs to modified to be more readable.

11) Page 13, line 375, why so high concentration of BSA is used here?

12) Page 14, line 379, more detailed information on the radioactive reaction solution is required.

13) In Figure Legends 3, and 6, "following" should be "followed".

14) Page 20-21, line 578-579, how is the "densitometry" done?

15) Figs 1B, 2G, 3A, 3C, 6B, the error bars are missing. Also, all the bar graphs need to have the statistical labels. All the images need to have scale bars. The text size of the labels in all the figure need to be large enough for visibility.

16) Fig 6D and E could be combined.

First revision

Author response to reviewers' comments

We appreciate your helpful suggestions on improving our manuscript. We have revised our manuscript accordingly taking into consideration all of the comments. Please see our point-to-point responses to the comments below.

To Reviewer 1: We would like to thank you for your positive comments and constructive suggestions. We appreciate all of your suggestions which we have addressed below to improve the quality of our manuscript.

1.Introduction. Is a little long and rambling. It could be made shorter and only include information explaining role of APC-FZR1 in mouse oocytes, and the examination here of role of CENP-T in regulating its activity. Much of the extra information could be incorporated into the Discussion.
Response: Thank you for this suggestion. As suggested, we made short of the Introduction part to introduce the background of our experiment.

2.All figures. State how many repeats in the figure legend for each experiment
Response: Thank you for this helpful comment. We have revised the figures as suggested.

3.Blots. All of the western blots in the paper are highly cropped. This is ok for presentation style. I would suggest for complete disclosure best to have full blots given in SI.
Response: We appreciate your comment. We provided our original full blots for all the western blots in supplemental data as suggested.

4.Fig 2. Using SEM here is not really informative. I'd like to know the spread of the data. Suggest using box plots or using sd. Fig 2D is particularly uninformative as displayed, and suggest to add some annotation to it.
Response: Thank you for your suggestion. Sorry that we do not understand your question. Fig 2D is a result for western, and the relative staining intensity was assessed by densitometry, we used SEM for data analysis in Figure 2 B and Figure 2 H to show the percentage of GVBD in each test. Because Figure 2H contains 4 different groups of injected oocytes we prefer to use mean value to make it easier to be understood instead of show each data of each experiment. Thank you again for your comment.

5.Line 128. "Thus, CenpT depletion causes prophase I arrest of oocytes by inhibiting MPF activity." Not strictly correct. More correctly: The arrest is associated with lack of MPF activity caused by loss of cyclin B1 and lack of CDK1 dephosphorylation.
Response: Thank you for your great advice. We have revised the text as follows: "Thus, CenpT depletion leads to prophase I arrest of oocytes, which is caused by loss of cyclin B1 and lack of CDK1 dephosphorylation, further leading to decreased MPF activity."

6."negative control MO"…. Detail needed in Materials and Methods. Currently no sequence is given.
Response: Thank you for this important comment. We revised our text, and added the sequence of negative MO used as control in our test.

7.Statistical analysis. Much of the data presented involves a comparison of just two groups (with or without MO for example). The Methods state ANOVA was used. However in these instances surely another test is being used? Also the posthoc test applied for ANOVA is a little unusual. Better to use Tukeys, which is generally regarded as being less liable to giving a Type 1 error.
Response: Thank you for your great advice. We preferred to compare only two groups of relative density for western blot and Time-lapse live imaging experiments, because we only showed once result in our text, however, each experiment was repeated at least three times. At for the rest data including the percentage of PB1 extrusion or GVBD, we actually use Tukeys for data analysis.

8."We also found that oocytes at the MII stage showed increased percentages of abnormal chromosome distribution in CenpT mRNA injected oocytes (Fig. 5D, E)". I find the terminology of abnormal chromosome distribution to be vague and the figures unhelpful in clarifying what is considered normal. Authors could be more descriptive of what abnormalities are being seen and how they are objectively being scored.
Response: Thank you for your excellent comment. We have added the description of normal and abnormal chromosome distribution in our text.

9.Was there reference to the SI movies in the manuscript? In any case their inclusion is probably unneeded. They are currently poor resolution brightfield timelapses that are not so informative.
Response: We appreciate your great comment. We have improved our data for time-lapse live imaging experiment, and we added the SI movies.

10.Discussion. I do not see need to reference figures again in the text. It almost reads as a repeat of the Results. Authors should move references to figures.

Response: Thank you for your important comment. We have revised the discussion part.

11. There are many small typos and badly phrased sentences in the submission. For example: Line 110 GV stage were collected form female mice at six weeks of age. Should be 'from'.
Response: Thank you for your comment. We have revised the text and edit the language.

To Reviewer 2: Thank you very much for your positive comments and valuable suggestions. We appreciate all of your helpful suggestions on improving the quality of our manuscript.

1. No specific scientific question raised by the researchers has been shown in either Abstract or Introduction part.
Response: Thank you for your important comment. We have added the question in the introduction part.

2. The Discussion part looks more like a duplicate of the major results, rather than a well organized discussion of the major conclusion of the research. Therefore, the authors needs to rewrite the part.
Response: Thank you for your important comment. We have extensively rewriten the discussion part.

3. The illustrations in several figures are not accurate or lack of detailed information, which includes Fig.3 E, Fig.4 CD, Fig.5 A and Fig.6 DE. Please refine them.
Response: Thank you so much for your advice. We have revised the figures.

4. The statistic significance indication are not unanimously marked as the rest figures, such as Fig.5 D, Fig. 6 ADE and Fig. 7E, which did not have lines to indicates the differences between the control and the experimental group.
Response: Thank you for your important comment. We have added the lines to indicates the differences in Figure 4D, Figure 5E. As for Figure 5A and Figure 5D, we do not add lines because there is no significant difference between the control group and experimental group.

5. The fonts of the words within the manuscript are not consistant.
Response: Thank you for your great comment. We have revised the text.

To Reviewer 3: Thank you very much for your positive comments and valuable suggestions. We appreciate all of your helpful suggestions on improving the quality of our manuscript.

Major concerns:
1) The major weakness of this study is the lack of mechanism that link CENPT and CDH1. How is the function of CENPT connected to CDH1? Is it a direct interaction between CENPT and CDH1?
Response: Thank you for your comment. We added a coimmunoprecipitation experiment to find whether CDH1 could direct interaction with CENPT, and the results showed yes.

2) The function of CENPT in the control of oocyte meiotic progression needs to be tested more vigorously by knockdown of CENPT in GVB oocytes. Acute deletion of CENPT in GVB oocytes by TrimAway method is highly recommended.
Response: We appreciate your comment. We tried to inject CenpT antibody to knockdown CenpT in GVBD oocytes, however, we failed to make it because of the limit of antibody, and thus it will be hard to specifically knockdown CenpT even by TrimAway method. TriAway depends much on the effective antibody, and we failed to make it work. Thank you for your advice again.

3) In the GV stage, knockdown of CENPT caused the upregulation of CDH1 that in turns reduced the levels of CCNB1 and MPF activity; while in GVB oocytes, over-expression of CENPT lowered down the levels of CDH1, but CCNB1 was also prematurely degraded. How did this happen? Does this mean that there is different mechanism(s) operating at GV stage and MI-anaphase I transition? Is CDC20 prematurely activated? Is securin also prematurely degraded?
Response: We appreciate your comment. We demonstrated that CENPT could mediate CDH1, and upregulation of CDH1 then lead to CCNB1 degraded at GV stage; as for MI-anaphase I transition,

APC complex work with CDC20 instead of only CDH1 at GV stage, which cause CCNB1 prematurely degraded. We tested the change of CDC20 and securin, and found thst CDC20 is activated during MI-anaphase I transition in both control oocytes and CENPT overexpressed oocytes. There was no obvious change for securin expression.

4) A more focused and succinct Discussion is required. Please get rid of the redundant repeat of the results in the Discussion.
Response: Thank you for your important comment. We have rewriten the discussion part.

5) A diagram is suggested to demonstrate the major findings of this study.
Response: Thank you for your important comment. We have added a diagram to show the major findings.

6) The upregulation of CENPT at GVB stage is very intriguing. Is it due to the activation of translation? What is the potential function of this dramatic increase? This needs to be discussed. Also, should this increase be reflected by the IF staining?
Response: Thank you for your important advice. We think the upregulation of CENPT at GVBD stage is actually due to activation of translation. The dramatic increase of CENPT at GVBD stage could might be a trace to tell that CENPT might have another function except just work as a kinetochore protein at GVBD stage, such as regulation of CDH1 which is mentioned in our experiment. Regretfully, this increase could not be reflected by IF staining because of the limit of antibody, we could not find a suitable antibody to stain CENPT in oocytes effectively, and we stain CENPT in oocytes by plasmid construction.

Some minor issues:
1) Standard nomenclature of the mouse genes, mRNAs, and proteins are highly recommended.
Response: Thank you for your great comment. We have revised the text.

2)The format of the reference needs to be carefully checked.
Response: We appreciate your great comment. We have revised the reference part.

3)Page 2, line 56-57, this is an awkward sentence.
Response: Thank you for your comment. We have revised the text.

4) Page 3, line 68-70, this sentence needs to be modified. Line 77, What is "T-terminal"? Line 77-84, this part need to be modified.
Response: Thank you for your advice. We have revised the text.

5) Page 4, line 88, "the meiotic cell cycle" is highly recommended to be changed into "meiotic progression". The same as to line 98. Line 91, Is it a real "continuous degradation"?
Response: We appreciate your comment. We have revised the text.

6) Page 4, line 114, How about extend the culture up to 20h?
Response: Thank you for your comment. We think 3 hours are enough for oocytes to undergo GVBD. However, we observed the oocytes till 14 hours after release from IBMX, up to 80% oocytes, which underwent GVBD at 3 hours, the first polar body has been extruded; as for the rest up to 90% oocytes were still stay at GV stage.

7) Page 5, line 130, "low levels of cyclin B1"≠ "cyclin B1 malfunction".
Response: We appreciate your comment. We have revised the text.

8) Page 6, line 147, "collecting them" should be "collected". Line 151, "translation" should be "transition". Line 150-170, this part needs to be modified, paying attention to use passive tense, and the results need to stated precisely.
Response: Thank you for your comment. We have revised the text.

9) Page 7, line 174, what does "realized" mean here?
Response: Thank you for your important comment. We have replaced “realized” with “achieved”.

10) Page 8, line 213-215, this sentence needs to modified to be more readable.

Response: Thank you for your comment. We have revised the text.

11) Page 13, line 375, why so high concentration of BSA is used here?
Response: Thank you for your important comment. There are two main reasons for using such high concentration of BSA. The first reason is to provide a very hypotonic environment that accelerates disruption of the oolemma; the second one is to stabilize kinase activity following lysis (Kubiak, 2011).
Reference: Kubiak, J. Z.. (2011). Protein kinase assays for measuring MPF and MAPK activities in mouse and rat oocytes and early embryos. Methods Mol Biol, 957, 77-89.

12) Page 14, line 379, more detailed information on the radioactive reaction solution is required.
Response: Thank you for your great advice. We have added the contents of HK buffer and radioactive reaction solution.

13) In Figure Legends 3, and 6, "following" should be "followed".
Response: Thank you for your advice, but we prefer to use “following”.

14) Page 20-21, line 578-579, how is the "densitometry" done?
Response: Thank you for your comment. For Figure 6B, we circled the oocytes, and the densitometry is spontaneously measured by Ultra VIEW VOX Confocal Imaging System.

15) Figs 1B, 2G, 3A, 3C, 6B, the error bars are missing. Also, all the bar graphs need to have the statistical labels. All the images need to have scale bars. The text size of the labels in all the figure need to be large enough for visibility.
Response: Thank you for your advice. In Figure 2G and 6B, the data come from fluorescence intensity for only one test we showed in Figure 2F and 6A to show the change of cyclin B1, so no bar is needed. For the rest of them, we added bars for each figure, and we improved our figure quality.

16) Fig 6D and E could be combined.
Response: Thank you for your comment. We prefer to separate these two results to show it clearly, but we have added a bar graph Fig. F to better compare the CDH1 level of control oocytes with that of experimental oocytes.

Second decision letter

MS ID#: JOCES/2019/238105

MS TITLE: CENP-T, regulates both G2/M transition and anaphase entry by acting through CDH1 in meiotic oocytes

AUTHORS: Yue Wang, Jian Li, Feng Dong, Wei Yue, Yin-Chun Ouyang, Zhen-Bo Wang, Yi Hou, Heide Schatten, and Qing-Yuan Sun
ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks.

Reviewer 1

Advance summary and potential significance to field

I am satisfied with the corrections made by the authors and suggest the paper is appropriate for publication.

Comments for the author

I am satisfied with the corrections made by the authors and suggest the paper is appropriate for publication.

Reviewer 2

Advance summary and potential significance to field

In this study, the novel function of CENP-T in oocyte meiosis is uncovered by a serial assays which emphasis on its action on regulating CDH1 level. The action of CENP-T in oocyte meiosis is greatly different from that in somatic cells. In my opinion, the findings in this study is interesting and have provided new clues to better understand the detailed meiosis process.

Comments for the author

I am satisfied with the answers provieded by the authors. I have no further questions. The manuscript is now acceptable for publication in JCS.

Reviewer 3

Advance summary and potential significance to field

The finding of the role of CENPT in the control of oocyte meiotic resumption and progression is new, which will substantiously prompt studies in this field.

Comments for the author

The manuscript is significant improved.
There are 2 minor issues needed to be taken care of:
1. The comma following CENPT in the title could be removed.
2. The standard nomenclature of CENPT is not CENP-T.