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MYCBPAP is a central apparatus protein required for centrosome-nuclear envelope docking and sperm tail biogenesis in mice

Haoting Wang, Hiroko Kobayashi, Keisuke Shimada, Seiya Oura, Yuki Oyama, Hiroaki Kitakaze, Taichi Noda, Norikazu Yabuta, Haruhiko Miyata and Masahito Ikawa
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Original submission

First decision letter

MS ID#: JOCES/2024/261962

MS TITLE: MYCBPAP is a central apparatus protein required for centrosome-nuclear envelope docking and sperm tail biogenesis in mice

AUTHORS: Haoting Wang, Hiroko Kobayashi, Keisuke Shimada, Seiya Oura, Yuki Oyama, Hiroaki Kitakaze, Taichi Noda, Norikazu Yabuta, Haruhiko Miyata, and Masahito Ikawa
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 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. I would encourage you to address point 5 of reviewer 3, who asks for immunolocalisation of FLAG- MYCBPAP as this will definitely strength the impact of your study. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript.

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

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This study generated a Mycbpap knockout (KO) mouse model and revealed the function of MYCBPAP in male fertility and spermiogenesis. This study demonstrated the essential roles of MYCBPAP in centrosome-nuclear envelope docking and sperm tail biogenesis. Though the manuscript highlights some new findings, the authors should address key issues to make the manuscript for publication.

Comments for the author

1. There are some language errors in the text that need careful correcting the spelling, word use throughout this manuscript.
2. The quantity of spermatozoa in the cauda epididymis of Mycbpap+/+ and Mycbpap-/- mice needs to be quantified in Fig. 3A.
3. Lines 170-172: Due to the fact that Mycbpap-/- males displayed abnormal head morphology after step 9, the flawed head shaping was likely linked to the abnormally long manchette rather than the abnormal removal of the manchette.
4. The interaction between MYCBPAP and CP110 is suggested for verification.
5. To demonstrate the role in centrosome docking, it is suggested to detect the localization of centrosome proteins such as CETN1/2 or CP110 in Mycbpap-/- spermatids.

Reviewer 2

Advance summary and potential significance to field

The authors generated Mycbpap knockout mice and demonstrated the essential role of Mycbpap in male fertility. Deletion of Mycbpap led to disrupted centrosome-nuclear envelope docking and abnormal flagellar biogenesis. Their findings provide insights into a MYCBPAP-dependent regulation of the centrosome-nuclear envelope docking and sperm tail biogenesis.

Comments for the author

There are several problems:
1. In Figure 2B, what was the p value? Since there were only three data points for each group, the author can add the actual three data points.
2. In Figure 3B, why the variance of the second group were so much greater the first group?
3. In Figure 5D, why there were much more upregulated genes? Have the data be properly normalized?
4. In Figure 5F, have the authors done multiple test adjustment?
5. The authors should add a summary mechanism figure to show how their findings were connected with previous knowledge.

Reviewer 3

Advance summary and potential significance to field

Wang et al characterise a novel protein, MYCBPAP, and demonstrate its essential function for male fertility. They use genome editing to disrupt the locus of the MYCBPAP gene, and demonstrate that the loss of this protein leads to immotile spermatozoa that display short flagella which impedes directional swimming, thus leading to male infertility.

Comments for the author

The manuscript is written in a clear, easy-to-follow style, figures are of high quality and allow an easy understanding of the results.
There are a large number of proteins that play important roles in the formation of motile flagella, and even small defects in flagellar structure can lead to defects in their function. In mammals, one of the key functions of flagella is to propel spermatozoids to the oocyte for fertilisation. So far, many advances in the understanding of flagellar functions have been made in model organisms such

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as Chlamydomonas. It remains, however, essential to study the role of proteins discovered in model organisms in mammals. The current paper thus undertook the endeavour to study the role of the protein FAP147, first described as a central-apparatus protein in Chlamydomonas flagella by electron microscopy, in mice. This is, to the knowledge of this reviewer, the first functional study of this protein in any model organism, and thus has the novelty and interest required for publication in the Journal of Cell Science.
Nonetheless, the current manuscript has some weaknesses listed below that must be addressed.

Major points:

1) In Fig. 2, the authors show first an overview of the testes of Mycbpap mutant mice. The histology in panel C clearly shows an absence, or strong reduction, of mature sperm nuclei. However, in panel D, the authors show for each step of spermatogenesis an example for wild-type and a Mycbpap mice. While these photos clearly show that there are defects starting from step 9, this panel hides the most striking phenotype: the almost complete absence of mature sperm heads. The authors should thus complement this figure by a statistical analysis of how many of each nuclei they can count in a given seminiferous tubule.

2) Fig 3C: the authors should show, in the supplement, the analyses of the 3 mice separately. In the plot shown in the main figure, it would be nice to colour-code the single data points for the 3 different mice, which would allow to appreciate whether (or not) the phenotype was the same in all 3 mice.

3) Similar to the problem mentioned in point (1), the authors must quantify the phenotypes shown in Fig 3D to make a compelling point about the abnormal timing of manchette removal. If this referee understands the argument the authors try to make, one would expect a predominance of manchettes shown in the left-most panel of the Mycbpapem1/em1 panel.

4) It was not clear to this reviewer whether the statement “MYCBPAP is shown to participate in not only the cilium movement but also the assembly of dyneins and cilia.” is the conclusion of the proteomic analysis, or whether the author refer to something else. If it is the conclusion of the proteomics, then it is an overstatement, as the interactions they find suggest so, but do not prove the fact. In case the statement refers so a known fact in the literature, then the paper should be cited.

5) The manuscript provides several lines of evidence that suggest a direct localisation of the protein MYCBPAP at the central apparatus of the axoneme, and suggest it might also play a key role at the centrosome. Given that they have a FLAG- MYCBPAP mouse, it is regrettable that they did not use FLAG antibodies in immuno-EM to show the localisation of the protein. Doing these experiments would strongly improve the manuscript.

Minor points:

1) Fig. 1A,B lack molecular weight markers

2) In Fig. 1F and 5A, the authors should use a scatter plot rather than a bar graphs with error bars.

3) Plots in all figures: instead of indicating statistic tests with stars, the authors could put p-values directly as well.

4) Suppl. Fig S2: the colours used in the panels should be directly indicated in the figure, not only in the legend, for easier reading.

5) Fig S2A: could the authors provide statistics here? How many apoptotic cells per seminiferous tubule? This would make a strong point and could move this panel to the main figure.

6) PNA (suppl. Fig. S2A) is not explained in the text.

7) The authors need to explain what IZUMO1 and BASIGIN stands for.

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8) In the discussion: “Further, we displayed that…” sounds unusual, perhaps replace “displayed” with “demonstrated”?

First revision

Author response to reviewers' comments

We thank the editors and reviewers for their careful reading and thoughtful comments that helped us improve our study. We wrote responses to reviewers’ comments in black with the original reviewers’ comments in blue. We highlighted all changes made in the revised manuscript.

Reviewer 1 Advance Summary and Potential Significance to Field:
This study generated a Mycbpap knockout (KO) mouse model and revealed the function of MYCBPAP in male fertility and spermiogenesis. This study demonstrated the essential roles of MYCBPAP in centrosome-nuclear envelope docking and sperm tail biogenesis. Though the manuscript highlights some new findings, the authors should address key issues to make the manuscript for publication.

Thank you very much for your comments.

Reviewer 1 Comments for the Author:
1. There are some language errors in the text that need careful correcting the spelling, word use throughout this manuscript.

We corrected the manuscript with a native English speaker.

2. The quantity of spermatozoa in the cauda epididymis of Mycbpap+/+ and Mycbpap-/- mice needs to be quantified in Fig. 3A.

We have quantified the number of cauda epididymal spermatozoa for *Mycbpap* heterozygous and homozygous mice and incorporated the results in Fig. S2B. A sentence explaining this result was added in lines 158-159.

3. Lines 170-172: Due to the fact that Mycbpap-/- males displayed abnormal head morphology after step 9, the flawed head shaping was likely linked to the abnormally long manchette rather than the abnormal removal of the manchette.

We have revised this sentence (lines 172-173 and 175-176).

4. The interaction between MYCBPAP and CP110 is suggested for verification.

We have verified the interaction between MYCBPAP and CCP110 by Co-IP and incorporated this result in Fig. 5E and lines 266-267.

5. To demonstrate the role in centrosome docking, it is suggested to detect the localization of centrosome proteins such as CETN1/2 or CP110 in Mycbpap-/- spermatids.

We attempted immunohistochemistry of centrosomes with an anti-CCP110 antibody but unfortunately encountered difficulties. However, from Western blot analysis no reduction in the amount of CCP110 in *Mycbpap*$^{-/-}$ testes (Fig. S4E) was detected, suggesting that centrosomes are not affected in *Mycbpap*$^{-/-}$ testis. We mentioned it in lines 267-269.

*****

Reviewer 2 Advance Summary and Potential Significance to Field:
The authors generated Mycbpap knockout mice and demonstrated the essential role of Mycbpap in

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male fertility. Deletion of Mycbpap led to disrupted centrosome-nuclear envelope docking and abnormal flagellar biogenesis. Their findings provide insights into a MYCBPAP-dependent regulation of the centrosome-nuclear envelope docking and sperm tail biogenesis.

Thank you very much for your comments.

Reviewer 2 Comments for the Author: There are several problems:
1. In Figure 2B, what was the p value? Since there were only three data points for each group, the author can add the actual three data points.

We indicated the p-value directly on the figure and changed the figure to a dot plot.

2. In Figure 3B, why the variance of the second group were so much greater the first group?

Tail lengths in *Mycbpap* KO may have a larger range due to abnormal sperm flagellum elongation. We modified Fig. 3C to better represent the variation in tail length in each male.

3. In Figure 5D, why there were much more upregulated genes? Have the data be properly normalized?

In Fig. 5D, we immunoprecipitated MYCBPAP-FLAG with an anti-FLAG antibody and analyzed interacting proteins. It is likely that there are more upregulated proteins in Tg testes because there is no *Mycbpap*-FLAG expression in WT testes. We have included more text in the figure for better clarification. We also added explanations in the figure legend.

4. In Figure 5F, have the authors done multiple test adjustment?

We performed multiple test adjustments using the Benjamini-Hochberg method and replaced Fig. 5F.

5. The authors should add a summary mechanism figure to show how their findings were connected with previous knowledge.

We have summarized our findings in Fig. 5G.

*****

Reviewer 3 Advance Summary and Potential Significance to Field:
Wang et al characterise a novel protein, MYCBPAP, and demonstrate its essential function for male fertility. They use genome editing to disrupt the locus of the MYCBPAP gene, and demonstrate that the loss of this protein leads to immotile spermatozoa that display short flagella which impedes directional swimming, thus leading to male infertility.

Thank you very much for your comments.

Reviewer 3 Comments for the Author:
The manuscript is written in a clear, easy-to-follow style, figures are of high quality and allow an easy understanding of the results.
There are a large number of proteins that play important roles in the formation of motile flagella, and even small defects in flagellar structure can lead to defects in their function. In mammals, one of the key functions of flagella is to propel spermatozoids to the oocyte for fertilisation. So far, many advances in the understanding of flagellar functions have been made in model organisms such as Chlamydomonas. It remains, however, essential to study the role of proteins discovered in model organisms in mammals. The current paper thus undertook the endeavour to study the role of the protein FAP147, first described as a central-apparatus protein in Chlamydomonas flagella by electron microscopy, in mice. This is, to the knowledge of this reviewer, the first functional study of this protein in any model organism, and thus has the novelty and interest required for publication in the Journal of Cell Science.
Nonetheless, the current manuscript has some weaknesses listed below that must be addressed.

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We appreciate your comments.

Major points:
1) In Fig. 2, the authors show first an overview of the testes of Mycbpap mutant mice. The histology in panel C clearly shows an absence, or strong reduction, of mature sperm nuclei. However, in panel D, the authors show for each step of spermatogenesis an example for wild-type and a Mycbpap mice. While these photos clearly show that there are defects starting from step 9, this panel hides the most striking phenotype: the almost complete absence of mature sperm heads. The authors should thus complement this figure by a statistical analysis of how many of each nuclei they can count in a given seminiferous tubule.

It is difficult to count the number of spermatozoa on testicular sections accurately due to different angles of each section. Instead, we counted the number of spermatozoa obtained from the cauda epididymis and incorporated the result into Fig. S2B. We also incorporated images of testicular sections in Fig. S2A, which indicate the decreasing number of sperm heads during spermiogenesis and added the explanations in lines 143-148.

2) Fig 3C: the authors should show, in the supplement, the analyses of the 3 mice separately. In the plot shown in the main figure, it would be nice to colour-code the single data points for the 3 different mice, which would allow to appreciate whether (or not) the phenotype was the same in all 3 mice.

We color-coded Fig. 3C to show that the short tail phenotype was similar in all 3 mice.

3) Similar to the problem mentioned in point (1), the authors must quantify the phenotypes shown in Fig 3D to make a compelling point about the abnormal timing of manchette removal. If this referee understands the argument the authors try to make, one would expect a predominance of manchettes shown in the left-most panel of the Mycbpapem1/em1 panel.

Because spermatogenic cells were squeezed out from seminiferous tubules, these cells were mixed and it is difficult to count the number of cells with abnormal manchettes at each stage. As Reviewer 1 mentioned (comment 3), flawed head shaping may be linked to the abnormally long manchette rather than the abnormal removal of the manchette. We discussed this possibility in lines 172-173 and 175-176.

4) It was not clear to this reviewer whether the statement “MYCBPAP is shown to participate in not only the cilium movement but also the assembly of dyneins and cilia.” is the conclusion of the proteomic analysis, or whether the author refer to something else. If it is the conclusion of the proteomics, then it is an overstatement, as the interactions they find suggest so, but do not prove the fact. In case the statement refers so a known fact in the literature, then the paper should be cited.

It was an overstatement and we have revised this point (lines 270-274).

5) The manuscript provides several lines of evidence that suggest a direct localisation of the protein MYCBPAP at the central apparatus of the axoneme, and suggest it might also play a key role at the centrosome. Given that they have a FLAG- MYCBPAP mouse, it is regrettable that they did not use FLAG antibodies in immuno-EM to show the localisation of the protein. Doing these experiments would strongly improve the manuscript.

Unfortunately, our immuno-TEM does not have enough resolution to separate the central apparatus and radial spoke as they are localized close to each other (Zhang et al, *Journal of Cell Science*, 2021; PMID = 34585727; Fig. 4). Therefore, we performed microtubule sliding, which separates microtubule doublet bundles with associated structures. MYCBPAP-FLAG signals were not found in all the separated doublet bundles, suggesting that MYCBPAP is localized in the central pair. We incorporated the result in Fig. S5 and discussed it in lines 249-259.

Minor points:
1) Fig. 1A,B lack molecular weight markers

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We have added molecular weight markers.

2) In Fig. 1F and 5A, the authors should use a scatter plot rather than a bar graphs with error bars.

We have revised this point.

3) Plots in all figures: instead of indicating statistic tests with stars, the authors could put p-values directly as well.

We added p-values in all plots.

4) Suppl. Fig S2: the colours used in the panels should be directly indicated in the figure, not only in the legend, for easier reading.

We indicated the colors used in the figure.

5) Fig S2A: could the authors provide statistics here? How many apoptotic cells per seminiferous tubule? This would make a strong point and could move this panel to the main figure.

We counted the percentage of seminiferous tubules that contain TUNEL-positive spermatids. The result was incorporated as Fig. S3A along with its explanation in lines 186-190. We have moved Fig. S2A to the main figure (Fig. 4C).

6) PNA (suppl. Fig. S2A) is not explained in the text.

We have revised this point (Fig. S3B legend).

7) The authors need to explain what IZUMO1 and BASIGIN stands for.

We have revised this point (lines 785-787 and 789).

8) In the discussion: “Further, we displayed that…” sounds unusual, perhaps replace “displayed” with “demonstrated”?

We have revised this point (line 283).

Second decision letter

MS ID#: JOCES/2024/261962

MS TITLE: MYCBPAP is a central apparatus protein required for centrosome-nuclear envelope docking and sperm tail biogenesis in mice

AUTHORS: Haoting Wang, Hiroko Kobayashi, Keisuke Shimada, Seiya Oura, Yuki Oyama, Hiroaki Kitakaze, Taichi Noda, Norikazu Yabuta, Haruhiko Miyata, and Masahito Ikawa
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 publication integrity checks.

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