Cep57 and Cep57l1 function redundantly to recruit the Cep63-Cep152 complex for centriole biogenesis

Huijie Zhao, Sen Yang, Qingxia Chen, Xiaomeng Duan, Guoqing Li, Qiongping Huang, Xueliang Zhu and Xiumin Yan
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MS TITLE: Cep57 and Cep57l1 cooperate to recruit the Cep63-Cep152 complex for centriole biogenesis

AUTHORS: Huijie Zhao, Sen Yang, Xiaomeng Duan, Qingxia Chen, Guoqing Li, Qiongping Huang, Xueliang Zhu, and Xiumin Yan
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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 manuscript by Zhao et al examines the functional overlap of Cep57 and Cep57L1 during centriole biogenesis. Various functions have been previously ascribed to Cep57, including interactions with the PCM, suppression of centriole disengagement/overduplication, and a the kinetochore during chromosomal segregation. However, Cep57 is also known to interact with Cep63 and Cep152, key components in the process of centriole duplication, although a knock down of Cep57 is not known to affect centriole biogenesis. In this well written manuscript and experimentally solid study, the authors provide evidence that this lack of phenotype is due to functional redundancy with a related protein Cep57L1. They show that a double knock down of Cep57 and Cep57L1 prevents the recruitment of Cep63 and Cep152 and a failure in centriole duplication in U2OS cells. Additional data are also provided that Cep57L1, like Cep57, is found at proximal base of the mother centriole, but not at the deuterosome, during centriole amplification in multiciliated cells. In addition, they carry out domain mapping in both Cep57 and Cep57l1 to determine what parts interact with Cep63 and mediates centriole localization.
While solid and a contribution, the manuscript is quite limited in scope. The authors do not explore the functional nature of the redundancy of Cep57 or Cep57L1 in multiciliated cells, either in terms of centriole amplification, or in basal body function. Nor do they examine whether Cep57L1, like Cep57, has additional functions in dividing cells. Thus, I am mildly enthusiastic about publication as is, since the manuscript presents a solid circumscribed finding, but am nonetheless concerned that its limited scope will have limited impact. I leave the final decision to the other reviewers and editors more familiar with standards expected for Journal of Cell Science.

Comments for the author

The gray insets shown in the 3D-SIM images throughout the paper are hard to follow, because they are not labelled. Maybe they follow some sort of pattern, but this requires a lot of thinking on the reader's part.

It is not clear why GFP-Cep57 is shown in mTECs, in addition to the endogenous Cep57 staining in Figure 2. The latter is less likely to be artifactual, and authors do not interpret the GFP-Cep57 localization, for example, microtubule bundling seems to be driven by overexpression of Cep57-GFP in U2OS cells but not in mTECs.

Reviewer 2

Advance summary and potential significance to field

In this study, Zhao and colleagues analyzed the recruitment of Cep63 and Cep162 by Cep57 and a newly identified paralog of Cep57 called Cep57L1, during centriole assembly in U2OS and mTEC cells. They first studied Cep57 localization during mTEC differentiation and showed that it is recruited at parental centrioles in a manner reminiscent of Cep63.
Cep57 forms a ring around the proximal end of parental centrioles but is absent from deuterosomes. In later stages, Cep57 is recruited to the proximal end of all newly formed centrioles. The authors next used co-IP and GST-pull down assays to map the interaction domains between Cep57 and Cep63 and the centriole targeting sites. They show that Cep57 is targeted to the centriole via its C-terminus and recruits Cep63 and then Cep152 in a hierarchical manner via its N-terminus. They then tested this in cells by generating Cep57 and Cep63 null U2OS cell lines. They show that Cep63 depletion impairs the recruitment of Cep152 but not of Cep57, as expected. However, the recruitment of Cep63 or Cep152 is affected by Cep57 depletion. The authors then searched for Cep57-related genes in the genomes of human and mouse and identified Cep57L1. Cep57L1 interacts with Cep63 via its N-terminus, like Cep57. Intriguingly, the same domain is required for centriole targeting, unlike Cep57 which localizes at centrioles via its C-terminus. A GFP-tagged version of Cep57L1 forms a ring around the proximal end of centrioles like Cep57, and behaves like Cep57 during mTEC differentiation. Zhao and colleagues then analyzed the effect of a simultaneous depletion of Cep57 and Cep57L1 and found that it strongly affects the recruitment of

Cep63 and Cep152, as well as centriole duplication. They propose that Cep57 and Cep57L1 cooperate to recruit Cep63 and Cep152 for centriole duplication.
Overall, the work is beautifully executed and brings important new insights in the molecular mechanisms underlying centriole duplication. The identification of Cep57L1 and the demonstration of its role along with Cep57 in recruiting Cep63 and Cep152 sheds light on a key step in this process, and I it will thus be of interest for many readers in the field and beyond.

Comments for the author

My main comment is that the differences in the behavior of Cep57 and Cep57L1 could have been explored further. The different abilities of the two paralogs to bind microtubules, or their different centriole targeting domains are indeed quite fascinating. For instance Watanabe and coworkers (2019, PMID: 30804344) recently showed that Cep57 can bind to pericentrin and that Cep57 depletion leads to PCM disorganization and precocious centriole disengagement during mitosis. Is this also true for Cep57L1?

Other comments
- The values obtained for the diameter of the rings formed by the different proteins are much higher than those found by Lukinavicius et al (2013) and Watanabe et al. (2019). This would indicate that the rings are much wider than the centrioles themselves, which is in contracts with the previous studies.
- I find Figure 1 a bit difficult to read as it is. The panels showing the localization of GFP-Cep57 do not add much information compared to panel E and could be shown as supplemental data. Also, the schematic representations of the different stages shown in Figure 1G could be placed next to the images to make them easier to understand.
- The N-terminal domain of Cep57L1 poorly localizes to centrioles compared to the full length protein, based on Fig3D. Could it be that the centriole targeting domain actually spans both the N- and C-terminal fragments?
- Quantitative information should be included for Fig. 4A-C. At the minimum, the numbers of cells observed for each condition should be indicated.
- The percentage of SAS-6 positive cells appear to be very high, as SAS-6 is not present in G1. Can the authors comment on that?
- Fig S2: It is surprising that in the CRISPR cell lines both copies of the CEP63 and CEP57 genes show the exact same mutation. Are the authors certain that both alleles have been sequenced in each case? A western blot showing the total levels of Cep57 and Cep63 in these cell lines would help convince that the clones are indeed null clones.

Reviewer 3

Advance summary and potential significance to field

This work shows a novel role for Cep57 proteins in the control of centriole biogenesis. Interestingly, the authors show that Cep57 and its paralog Cep57L1 function redundantly in the recruitment of Cep63-Cep152 to the proximal end of mother centrioles to control centriole replication. They also mapped the domains of the two Cep57 paralog proteins that directly interact with a domain within Cep63. The issue of paralog redundancy is seldom addressed in such studies and it is satisfying to see it executed convincingly here. While functionally redundant in recruitment of Cep63-Cep152, Cep57 and Cep57L1 show a difference with respect to centriole-targeting domains and their apparent ability to bind/bundle microtubules, suggesting that they may have different functions not discerned in this study.
Overall this is an excellent paper and the experimental results support the conclusions being made. A few minor issues should be addressed to clarify the presentation of the work and to provide quantitative rigor to some of the conclusions.

Comments for the author

Specific comments:
1. In the abstract, stating that Cep57 and Cep57L1 act together for procentriole assembly perhaps does not accurately reflect the key findings: “We propose that Cep57 and Cep57l1 act together to

ensure the recruitment of the Cep63-Cep152 complex to the mother centrioles for procentriole formation.". Instead since depletion of either gene had no impact on Cep63-Cep152 recruitment and only the double affected it, and since either could rescue this, it is more accurate to state that they function redundantly (not 'together' or 'cooperatively'). Thus, the title should be adjusted too.
2. The manuscript needs page numbers.
3. Introduction: There are multiple English usage corrections needed (not just in the introduction) in order to make the message clearer. For example, in the introduction, "In" should precede the sentence that starts with "Recent years, great progress…", and "mechanism" should be plural in that sentence. And in this sentence, I think the meaning is to understand how the Cep63-Cep152 complex is recruited to the mother centriole, and so it should be "targeted to" rather than "targeted at": "However, it is still unclear how Cep63 or the Cep63-Cep152 complex is targeted at the proximal end of mother centrioles".
Additionally, in this sentence, "the" should be replaced with "a", since there was more than one proximity interactor: "…and Cep57 is the proximity interactor of Cep63…". Also, the information in the paragraph leading up to this statement does not support the idea that Cep57 is upstream: "…suggesting that Cep57 may act as the upstream protein to target the Cep63-Cep152 complex for centriole duplication." (the data in the paper support this statement, so you just need to revise the text that supports this statement in the introduction).
4. A little bit more background on Cep57 should be included, in addition to the work by Lukinavicius et al 2013 showing that Cep57 forms a complex with Cep63 and Cep152, a key premise for the work here.
Additional published work showed that it associated with NEDD1 to control PCM organization and spindle pole integrity (Wu et al 2012), and recent findings showed that it is required with PCNT for centriole engagement and PCM organization (Watanabe et al 2019). A novel feature of the work here is inclusion of examination of Cep57l1, a paralog for which there is only one published paper (which should also be cited).
5. Figure 1A: The figure could probably be better represented without the main image as it is mostly empty space. The insets provide the relevant information and can be expanded into a larger image with four panels. In addition, it is difficult to tell which monograph refers to which signal. The authors should label each monochrome channel to indicate which signal it represents (Cep57, Cep63, or Cep157).
Otherwise, it is a challenge for the reader to figure out which monochrome image matches with which signal. Other images are difficult to interpret for the same reason that the channels are not labeled. Please label the monochrome images for Figures 1A, C-F, 2E-H, 3D,H,I, 4A-D, and S1A,C.
6. In Figure 1D the blue channel appears significantly overexposed or over-brightened compared to the overlay.
7. In the legend to Figure 1, "interfere" should be "interface".
8. "In addition to the centriolar localization, overexpression of GFP-Cep57 resulted in microtubule (MT) bundling and both Cep63 and Cep152 were recruited to the bundled MTs (Lin et al., 2013b; Momotani et al., 2008; Zhou et al., 2016) (Fig. 1C,D), indicating that Cep57, Cep63 and Cep152 form a complex and Cep57 could recruit Cep63 and Cep152 in vivo." I think this should be rephrased, because the point is not that Cep63 and Cep152 are recruited to MTs (which is not shown here), but rather that the filaments of Cep57 that form from their overexpression recruit these proteins.
9. Figure S2 and related data: Figure S2 has no text in the legend; what does "scr" in the figure stand for? In addition, the methods section describes how the CRISPR knockouts were achieved, but the authors should show western blot validation of the knockout cell lines of CEP57 and CEP63 as is standard practice. In addition, since 293T cells are likely diploid (or even higher ploidy), it is almost guaranteed that more than one mutant allele will be generated by CRISPR and the sequencing evaluation shown in Figure S2 only shows one allele for one clone for each, and it is unlikely that they are homozygous for the same lesion. It is OK to keep the data as is, but the authors should point this out (that one allele is represented in the sequencing data). The recommended practice is that multiple genomic PCR clones are sequenced for each clonal line in order to evaluate each allele (see doi:10.3791/52118). However, evaluation of the CRISPR knockouts by the loss or alteration of Cep57 or Cep63 protein expression by western blotting should suffice to demonstrate the knockouts in the cell lines used.
10. For the co-IP data presented in Figures 3C,F and S1B,D, please label the figure/blot with respect to what the IP was directed against (eg IP anti-GFP, etc) instead of just "IP". The info is in the legend, but it would be helpful to have it visually accessible in the figure.

11. In the legend to Figure 3I there is no graphic shown that corresponds to “Schematic illustration models are presented to aid understanding.” Add this in or, instead, maybe change the text to “see schematic illustration in Figure 1G III”?
12. For the CEP63 knockout U2OS cells, did the authors see any phenotypes that they can report? Others have reported aberrant centriole numbers and aberrant mitotic spindles resulting from CEP63 knockouts and it would be very beneficial to the community if the phenotypes can be described and compared in this case.
13. In the text of the legend to Figure 2, the Cep152 signal was not “abolished”; instead the authors should rephrase with “significantly reduced” or something to that effect. The same for the legend to Figure 4B. In addition, the authors should quantify the decreased recruitment of Cep152 to centrioles in the CEP63 and CEP57 knockout cells.
14. I think one of the panels in Figure 2F was meant to be a merge/overlay (CEP63 KO in G1).
15. On p. 8, the authors describe discovering “found out” Cep57l1 by BLAST homology search using Cep57 sequences, but CEP57L1 was previously known, and its disease association was described in a manuscript by Liu et al 2015, which should be cited here.
16. The legend for Figure S4 needs more info. All 8 lanes should be described or labelled, and the meanings of “NC” and “*” should be described or clarified. The footnote for “*” states “severe cell toxicity”, but it is not clear what this means or why GFP-Cep57L1 is affected differently from the other lanes whereas the loading control is not. If all the lanes except #3 and #8 are negative controls, why was the experiment designed this way? This experiment probably needs to be repeated as it is the essential validation of the CEP57L1 knockdowns, an important tool/reagent for this work.
17. In Figure 4, the authors should quantify the differences seen with recruitment of Cep63 and Cep152 to the control and CEP57 KO/CEP57L1 RNAi double depletion cells.
18. In Figure 4E, where centriole numbers are quantified in the CEP57 KO +/- CEP57L1 RNAi, what the “Ctrl” is should be stated in the legend. Also, it is unclear what significant differences the p values are representing. Please label the figure more clearly, indicating what comparisons the p values are representing. If there are no significant differences among cells with only one sas-6 dot, that should be included too.
19. The authors should rephrase this statement on p. 10, as it sounds like you are saying that Cep57 and Cep57L1 form a complex with each other, which I don’t think was the intended message: “In this study, we find that Cep57 and its paralog Cep57l1 form a complex and colocalize with the Cep63-Cep152 cradle at the proximal end of mother centrioles.”
20. Also, on p. 10, it is probably more accurate to say that Cep57 and Cep57L1 act redundantly to recruit the Cep63-Cep152 complex. Stating that they act cooperatively invokes a concerted mode of action that has not been demonstrated: “Our results indicate that Cep57 and Cep57l1 cooperate to recruit the Cep63-
Cep152 complex”.
21. The discussion regarding the role of Cep295 in centriole conversion should also include citation of the work by Izquierdo et al 2014.
22. Materials and Methods: need to fix the reagent source in this statement “…and complete protease inhibitors (Chemicals)) for 4 hours at 4oC. “ (ie specify ‘Chemicals’). This shows up in two sections of the M&M. Also, the authors should describe more specifically what parts of the two proteins were used to generate antibodies “Chicken anti-Cep152 (1:300) and Rat anti-Cep57 (1:200) polyclonal antibodies were generated with purified GST fused proteins (Abclonal). ”. The section on antibodies used for western blotting needs to include anti-GFP and anti-actin too.

First revision

Author response to reviewers' comments

Response to reviewers:

Reviewer 1

The manuscript by Zhao et al examines the functional overlap of Cep57 and Cep57L1 during

centriole biogenesis. Various functions have been previously ascribed to Cep57, including interactions with the PCM, suppression of centriole disengagement overduplication, and a the kinetochore during chromosomal segregation. However, Cep57 is also known to interact with Cep63 and Cep152, key components in the process of centriole duplication, although a knock down of Cep57 is not known to affect centriole biogenesis. In this well written manuscript and experimentally solid study, the authors provide evidence that this lack of phenotype is due to functional redundancy with a related protein Cep57L1. They show that a double knock down of Cep57 and Cep57L1 prevents the recruitment of Cep63 and Cep152 and a failure in centriole duplication in U2OS cells. Additional data are also provided that Cep57L1, like Cep57, is found at proximal base of the mother centriole, but not at the deuterosome, during centriole amplification in multiciliated cells. In addition, they carry out domain mapping in both Cep57 and Cep57l1 to determine what parts interact with Cep63 and mediates centriole localization. While solid and a contribution, the manuscript is quite limited in scope. The authors do not explore the functional nature of the redundancy of Cep57 or Cep57L1 in multiciliated cells, either in terms of centriole amplification, or in basal body function. Nor do they examine whether Cep57L1, like Cep57, has additional functions in dividing cells.
Thus, I am mildly enthusiastic about publication as is, since the manuscript presents a solid circumscribed finding, but am nonetheless concerned that its limited scope will have limited impact. I leave the final decision to the other reviewers and editors more familiar with standards expected for Journal of Cell Science.

Response:
We thank the reviewer for appreciating the quality of our manuscript.

In the revised manuscript, we examined Cep57l1-depleted mitotic U2OS cells and observed that they did not display PCM disorganization and premature centriole disengagement (Fig. S4C) as reported for Cep57 depletion (Watanabe et al., Nat Commun., 2019).

We agree that the functions of Cep57 and Cep57l1 in mTECs undergoing the centriole amplification are interesting issues. mTECs are mouse primary cells and require RNAi to deplete the proteins. Currently we still cannot find appropriate siRNAs capable of efficiently depleting mouse Cep57 and Cep57l1. Therefore, we hope that our reviewer could allow us to focus on human cycling cells and leave the issues in mTECs for future studies. In the revised manuscript, we have briefly discussed such issues.

Reviewer 1 Comments for the Author:

The gray insets shown in the 3D-SIM images throughout the paper are hard to follow, because they are not labelled. Maybe they follow some sort of pattern, but this requires a lot of thinking on the reader's part.

Response:
We have included color frames for the gray insets to aid comprehension.

It is not clear why GFP-Cep57 is shown in mTECs, in addition to the endogenous Cep57 staining in Figure 2. The latter is less likely to be artifactual, and authors do not interpret the GFP-Cep57 localization, for example, microtubule bundling seems to be driven by overexpression of Cep57-GFP in U2OS cells but not in mTECs.

Response:
Our reviewer appeared to refer to Figure 1 instead of Figure 2. Like in U2OS cells (Figure 1C,D), we used GFP-Cep57 to confirm the localization of the endogenous protein in mTECs. We have modified the main text so that the GFP-Cep57 localization is also interpreted.

We indeed did not observe bundled filaments in GFP-Cep57 overexpressing mTECs. As U2OS cells were transiently transfected and mTECs were infected with lentiviral particles, the difference in the bundled filament formation is likely due to different expression levels of GFP- Cep57.

Reviewer 2

In this study, Zhao and colleagues analyzed the recruitment of Cep63 and Cep162 by Cep57 and a newly identified paralog of Cep57 called Cep57L1, during centriole assembly in U2OS and mTEC cells. They first studied Cep57 localization during mTEC differentiation and showed that it is recruited at parental centrioles in a manner reminiscent of Cep63. Cep57 forms a ring around the proximal end of parental centrioles but is absent from deuterosomes. In later stages, Cep57 is recruited to the proximal end of all newly formed centrioles. The authors next used co-IP and GST-pull down assays to map the interaction domains between Cep57 and Cep63 and the centriole targeting sites. They show that Cep57 is targeted to the centriole via its C-terminus and recruits Cep63 and then Cep152 in a hierarchical manner via its N-terminus. They then tested this in cells by generating Cep57 and Cep63 null U2OS cell lines. They show that Cep63 depletion impairs the recruitment of Cep152 but not of Cep57, as expected. However, the recruitment of Cep63 or Cep152 is affected by Cep57 depletion. The authors then searched for Cep57-related genes in the genomes of human and mouse and identified Cep57L1. Cep57L1 interacts with Cep63 via its N-terminus, like Cep57. Intriguingly, thesame domain is required for centriole targeting, unlike Cep57 which localizes at centrioles via its C-terminus. A GFP-tagged version of Cep57L1 forms a ring around the proximal end of centrioles like Cep57, and behaves like Cep57 during mTEC differentiation. Zhao and colleagues then analyzed the effect of a simultaneous depletion of Cep57 and Cep57L1 and found that it strongly affects the recruitment of Cep63 and Cep152, as well as centriole duplication. They propose that Cep57 and Cep57L1 cooperate to recruit Cep63 and Cep152 for centriole duplication. Overall, the work is beautifully executed and brings important new insights in the molecular mechanisms underlying centriole duplication. The identification of Cep57L1 and the demonstration of its role along with Cep57 in recruiting Cep63 and Cep152 sheds light on a key step in this process, and I it will thus be of interest for many readers in the field and beyond.

Response:
We thank the reviewer for appreciating our work.

Reviewer 2 Comments for the Author:

My main comment is that the differences in the behavior of Cep57 and Cep57L1 could have been explored further. The different abilities of the two paralogs to bind microtubules, or their different centriole targeting domains are indeed quite fascinating. For instance, Watanabe and coworkers (2019, PMID: 30804344) recently showed that Cep57 can bind to pericentrin and that Cep57 depletion leads to PCM disorganization and precocious centriole disengagement during mitosis. Is this also true for Cep57L1?

Response:
We performed the suggested experiments and observed that neither the PCM organization nor the centriole disengagement was affected by the knockdown of Cep57l1. We have included these results in Supplementary Fig. S4C in the revised manuscript.

Other comments
-The values obtained for the diameter of the rings formed by the different proteins are much higher than those found by Lukinavicius et al (2013) and Watanabe et al. (2019). This would indicate that the rings are much wider than the centrioles themselves, which is in contracts with the previous studies.
Response:
We thank our reviewer for pointing out this difference. The values in the two papers are measured as the distance between the two peaks of the Gaussian curve, whereas the diameters we presented in the original manuscript were the outer diameter of each ring. To prevent misunderstanding, we re-quantified the diameters using their method.
Now the values (223 ± 31 nm for Cep57, 260 ± 25 nm for Cep63, and 371 ± 40 nm for Cep152) (Fig. 1B) are comparable to those in the published papers.

-I find Figure 1 a bit difficult to read as it is. The panels showing the localization of GFP-Cep57 do not add much information compared to panel E and could be shown as supplemental data. Also, the schematic representations of the different stages shown in Figure 1G could be placed next to the

images to make them easier to understand.

Response:
We have used up the allowed four supplemental figures for other data and found it inappropriate to incorporate the GFP-Cep57 data into these figures. We therefore still keep these data in Fig. 1. As suggested, we have placed the schematic representations next to the images in the revised manuscript.

- The N-terminal domain of Cep57L1 poorly localizes to centrioles compared to the full length protein, based on Fig3D. Could it be thatthe centriole targeting domain actually spans both the N- and C-terminal fragments?

Response:
We thank our reviewer for the comment. We re-examined the cell images and found that the centriolar localization of GFP-Cep57l1N is indeed weaker than that of GFP-Cep57l1. Additionally, GFP-Cep57l1C also displays very weak centriolar localization (Fig. 3D). We co-expressed RFP-Centrin1 with GFP-tagged Cep57l1 or its mutants and performed live imaging to double confirm this (Fig. S4A). Therefore, both the N- and C- terminal regions are required for the strong centriolar localization of Cep57l1. We have revised the text and the schematic accordingly.

- Quantitative information should be included for Fig. 4A-C. At the minimum, the numbers of cells observed for each condition should be indicated.

Response:
We have included the quantitative results in our revised manuscript.

- The percentage of SAS-6 positive cells appear to be very high, as SAS-6 is not present in G1. Can the authors comment on that?

Response:
This is because only S-phase (EdU-positive) cells were quantified in Figure 4E. We have modified the text to avoid confusion in the revised manuscript.

- Fig S2: It is surprising that in the CRISPR cell lines both copies of the CEP63 and CEP57 genes show the exact same mutation. Are the authors certain that both alleles have been sequenced in each case? A western blot showing the total levels of Cep57 and Cep63 in these cell lines would help convince that the clones are indeed null clones.

Response:
As requested, we have included immunoblotting results to show the complete depletion of Cep57 and Cep63 in the revised manuscript (Fig. 2E).

We also examined the mutation issue. We originally sequenced three independent clones of the PCR product for each KO line. During the revision we sequenced another three clones each line. For the *Cep57* KO cell line, all the six clones showed the same mutation. However, two of the new clones for the *Cep63* KO cell line showed a different mutation from the other four. Despite this, both mutations caused reading frameshift. We have updated the information in the revised manuscript (Fig. S2A-B).

Reviewer 3

This work shows a novel role for Cep57 proteins in the control of centriole biogenesis. Interestingly, the authors show that Cep57 and its paralog Cep57L1 function redundantly in the recruitment of Cep63-Cep152 to the proximal end of mother centrioles to control centriole replication. They also mapped the domains of the two Cep57 paralog proteins that directly interact with a domain within Cep63. The issue of paralog redundancy is seldom addressed in such studies and it is satisfying to see it executed convincingly here. While functionally redundant in recruitment of Cep63- Cep152, Cep57 and Cep57L1 show a difference with respect to centriole- targeting domains and their

apparent ability to bind/bundle microtubules, suggesting that they may have different functions not discerned in this study. Overall this is an excellent paper and the experimental results support the conclusions being made. A few minor issues should be addressed to clarify the presentation of the work and to provide quantitative rigor to some of the conclusions.

Response:
We thank the reviewer for appreciating our work and providing detailed comments that have helped us to substantially improve the manuscript.

Reviewer 3 Comments for the Author: Specific comments:
1. In the abstract, stating that Cep57 and Cep57L1 act together for procentriole assembly perhaps does not accurately reflect the key findings: “We propose that Cep57 and Cep57l1 act together to ensure the recruitment of the Cep63-Cep152 complex to the mother centrioles for procentriole formation.”. Instead, since depletion of either gene had no impact on Cep63-Cep152 recruitment and only the double affected it, and since either could rescue this, it is more accurate to state that they function redundantly (not ‘together’ or ‘cooperatively’). Thus, the title should be adjusted too.

Response:
We have modified the title, abstract, and main text according to the comment.

2. The manuscript needs page numbers.
Response:
We are sorry for this. Page numbers have been added in the revised manuscript.

3. Introduction: There are multiple English usage corrections needed (not just in the introduction) in order to make the message clearer. For example, in the introduction, “In” should precede the sentence that starts with “Recent years, great progress…”, and “mechanism” should be plural in that sentence. And in this sentence, I think the meaning is to understand how the Cep63-Cep152 complex is recruited to the mother centriole, and so it should be “targeted to” rather than “targeted at”: “However, it is still unclear how Cep63 or the Cep63-Cep152 complex is targeted at the proximal end of mother centrioles”. Additionally, in this sentence, “the” should be replaced with “a”, since there was more than one proximity interactor: “…and Cep57 is the proximity interactor of Cep63…”. Also, the information in the paragraph leading up to this statement does not support the idea that Cep57 is upstream: “…suggesting that Cep57 may act as the upstream protein to target the Cep63-Cep152 complex for centriole duplication.” (the data in the paper support this statement, so you just need to revise the text that supports this statement in the introduction).

Response:
We thank the reviewer for pointing out the grammar mistakes. We have corrected these mistakes in the revised manuscript.

4. A little bit more background on Cep57 should be included, in addition to the work by Lukinavicius et al 2013 showing that Cep57 forms a complex with Cep63 and Cep152, a key premise for the work here. Additional published work showed that it associated with NEDD1 to control PCM organization and spindle pole integrity (Wu et al 2012), and recent findings showed that it is required with PCNT for centriole engagement and PCM organization (Watanabe et al 2019). A novel feature of the work here is inclusion of examination of Cep57l1, a paralog for which there is only one published paper (which should also be cited).

Response:
Thanks for the advice. In the revised manuscript, we have included the suggested references.

5. Figure 1A: The figure could probably be better represented without the main image as it is mostly empty space. The insets provide the relevant information and can be expanded into a larger image with four panels. In addition, it is difficult to tell which monograph refers to which signal. The authors should label each monochrome channel to indicate which signal it represents (Cep57, Cep63, or Cep157). Otherwise, it is a challenge for the reader to figure out which monochrome image matches with which signal. Other images are difficult to interpret for the same reason that the channels are not labeled. Please label the monochrome images for Figures 1A, C-F, 2E-H, 3D,H,I, 4A-D, and S1A,C.

Response:
We apologize for not clearly presenting our images. In the revised manuscript, we have reorganized Figure 1A as suggested and added color frames to all the grayscale insets to aid comprehension.

6. In Figure 1D the blue channel appears significantly overexposed or over-brightened compared to the overlay.

Response:
In the revised manuscript, we have replaced the overexposed image with a more appropriate 3D-SIM image.

7. In the legend to Figure 1, "interfere" should be "interface".

Response:
We are sorry for the typo and have corrected it.

8. "In addition to the centriolar localization, overexpression of GFP-Cep57 resulted in microtubule (MT) bundling and both Cep63 and Cep152 were recruited to the bundled MTs (Lin et al., 2013b; Momotani et al., 2008; Zhou et al., 2016) (Fig. 1C,D), indicating that Cep57, Cep63 and Cep152 form a complex and Cep57 could recruit Cep63 and Cep152 in vivo." I think this should be rephrased, because the point is not that Cep63 and Cep152 are recruited to MTs (which is not shown here), but rather that the filaments of Cep57 that form from their overexpression recruit these proteins.

Response:
Thanks for the suggestion. We have rephrased the sentence.

9. Figure S2 and related data: Figure S2 has no text in the legend; what does "scr" in the figure stand for? In addition, the methods section describes how the CRISPR knockouts were achieved, but the authors should show western blot validation of the knockout cell lines of CEP57 and CEP63 as is standard practice. In addition, since 293T cells are likely diploid (or even higher ploidy), it is almost guaranteed that more than one mutant allele will be generated by CRISPR and the sequencing evaluation shown in Figure S2 only shows one allele for one clone for each, and it is unlikely that they are homozygous for the same lesion. It is OK to keep the data as is, but the authors should point this out (that one allele is represented n the sequencing data). The recommended practice is that multiple genomic PCR clones are sequenced for each clonal line in order to evaluate each allele (see doi:10.3791/52118). However, evaluation of the CRISPR knockouts by the loss or alteration of Cep57 or Cep63 protein expression by western blotting should suffice to demonstrate the knockouts in the cell lines used.

Response:
We thank our reviewer for the comments. As requested, we have included immunoblotting results to show the complete depletion of Cep57 and Cep63 in the revised manuscript (Fig. 2E).
We also examined the mutation issue. We originally sequenced three independent clones of the PCR product for each KO line. During the revision we sequenced another three clones each line. For the *Cep57* KO cell line, all the six clones showed the same mutation. However, two of the new clones for the *Cep63* KO cell line showed a different mutation from the other four. Despite this, both mutations caused reading frameshift. We have updated the information in the revised manuscript (Fig. S2A-B).

10. For the co-IP data presented in Figures 3C, F and S1B,D, please label the figure/blot with respect to what the IP was directed against (eg IP anti-GFP, etc) instead of just "IP". The info is in the legend, but it would be helpful to have it visually accessible in the figure.

Response:
Following the reviewer's suggestions, we have included the information in the revised figures.

11. In the legend to Figure 3I there is no graphic shown that corresponds to "Schematic illustration

models are presented to aid understanding."Add this in or, instead, maybe change the text to "see schematic illustration in Figure 1G III"?

Response:
We apologize for this mistake and have removed this sentence in the revised manuscript.

12. For the CEP63 knockout U2OS cells, did the authors see any phenotypes that they can report? Others have reported aberrant centriole numbers and aberrant mitotic spindles resulting from CEP63 knockouts and it would be very beneficial to the community if the phenotypes can be described and compared in this case.

Response:
We indeed observed slightly aberrant mitotic spindles and centriole numbers. We have included this information in Fig. S2C-E in the revised manuscript.

13. In the text of the legend to Figure 2, the Cep152 signal was not "abolished"; instead the authors should rephrase with "significantly reduced" or something to that effect. The same for the legend to Figure 4B. In addition, the authors should quantify the decreased recruitment of Cep152 to centrioles in the CEP63 and CEP57 knockout cells.

Response:
Following the suggestion, we have rephased it and have included the quantitative information in the revised manuscript.

14. I think one of the panels in Figure 2F was meant to be a merge/overlay (CEP63 KO in G1).
Response:
We apologize for this mistake and have fixed it in the revised manuscript.

15. On p. 8, the authors describe discovering "found out" Cep57l1 by BLAST homology search using Cep57 sequences, but CEP57L1 was previously known, and its disease association was described in a manuscript by Liu et al 2015, which should be cited here.

Response:
In the revised manuscript, we have included the suggested reference and rewritten this sentence.

16. The legend for Figure S4 needs more info. All 8 lanes should be described or labelled, and the meanings of "NC" and "*" should be described or clarified. The footnote for "*" states "severe cell toxicity", but it is not clear what this means or why GFP-Cep57L1 is affected differently from the other lanes whereas the loading control is not. If all the lanes except #3 and #8 are negative controls, why was the experiment designed this way? This experiment probably needs to be repeated as it is the essential validation of the CEP57L1 knockdowns, an important tool/reagent for this work.

Response:
We apologize for the confusion. Figure S4B describes our screen for siRNAs capable of efficiently knockdown Cep57l1. In the revised manuscript, we have described the experiment in more details in the figure legend to avoid confusing.

17. In Figure 4, the authors should quantify the differences seen with recruitment of Cep63 and Cep152 to the control and CEP57 KO/CEP57L1 RNAi double depletion cells.

Response:
We have included the quantitative information in the revised manuscript.

18. In Figure 4E, where centriole numbers are quantified in the CEP57 KO +/- CEP57L1 RNAi, what the "Ctrl" is should be stated in the legend. Also, it is unclear what significant differences the p values are representing.
Please label the figure more clearly, indicating what comparisons the p values are representing. If there are no significant differences among cells with only one sas-6 dot, that should be included too.

Response:
The label is actually “Ctrli” (i.e., the control siRNA), not “Ctrl”. In the quantification results, the comparisons are all against the corresponding Ctrli groups. We have clarified this in the figure legend in the revised manuscript. We have also indicated the cell groups showing no significant difference.

19. The authors should rephrase this statement on p. 10, as it sounds like you are saying that Cep57 and Cep57L1 form a complex with each other, which I don’t think was the intended message: “In this study, we find that Cep57 and its paralog Cep57l1 form a complex and colocalize with the Cep63-Cep152 cradle at the proximal end of mother centrioles.”

Response:
Our reviewer is correct. We do not intend to say that the two proteins form a complex. We have modified the sentence: “In this study, we find that Cep57 and its paralog Cep57l1 localize to the proximal end of parental centrioles and function redundantly to recruit the Cep63-Cep152 complex”.

20. Also, on p. 10, it is probably more accurate to say that Cep57 and Cep57L1 act redundantly to recruit the Cep63-Cep152 complex. Stating that they act cooperatively invokes a concerted mode of action that has not been demonstrated: “Our results indicate that Cep57 and Cep57l1 cooperate to recruit the Cep63-Cep152 complex”.

Response:
We have modified the manuscript following the request.

21. The discussion regarding the role of Cep295 in centriole conversion should also include citation of the work by Izquierdo et al 2014.
Response:
We have included the suggested reference.

22. Materials and Methods: need to fix the reagent source in this statement “…and complete protease inhibitors (Chemicals)) for 4 hours at 4oC. “ (ie specify ‘Chemicals’). This shows up in two sections of the M&M. Also, the authors should describe more specifically what parts of the two proteins were used to generate antibodies “Chicken anti-Cep152 (1:300) and Rat anti-Cep57 (1:200) polyclonal antibodies were generated with purified GST fused proteins (Abclonal). ”. The section on antibodies used for western blotting needs to include anti-GFP and anti-actin too.

Response:
We have included the requested information in the revised manuscript.

Second decision letter

MS ID#: JOCES/2019/241836

MS TITLE: Cep57 and Cep57l1 function redundantly to recruit the Cep63-Cep152 complex for centriole biogenesis

AUTHORS: Huijie Zhao, Sen Yang, Xiaomeng Duan, Qingxia Chen, Guoqing Li, Qiongping Huang, Xueliang Zhu, and Xiumin Yan
ARTICLE TYPE: Short Report

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 gave favourable reports but raised some critical points that will require amendments to your manuscript. I hope that you will be able to carry these out, because I would like to be able to accept your paper.

We are aware that you may currently be unable to access the lab to undertake experimental revisions. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

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

This is a solid contribution describing the redundant functions of Cep57 and Cep57L1 in centriole biogenesis, and will be of interest to cell biologists working on centrioles.

Comments for the author

The authors have adequately addressed the concerns of the reviewers. My only remaining concern is that the authors need to carefully go over the figures and text to check for grammatical errors and English usage. As one example, in Figure 3B, the word mediate is used for intermediate, which is an unusual choice.

Reviewer 2

Advance summary and potential significance to field

In this study, Zhao and colleagues analyzed the recruitment of Cep63 and Cep162 by Cep57 and a newly identified paralog of Cep57 called Cep57L1, during centriole assembly in U2OS and mTEC cells. They first studied Cep57 localization during mTEC differentiation and showed that it is recruited at parental centrioles in a manner reminiscent of Cep63.
Cep57 forms a ring around the proximal end of parental centrioles but is absent from deuterosomes. In later stages, Cep57 is recruited to the proximal end of all newly formed centrioles. The authors next used co-IP and GST-pull down assays to map the interaction domains between Cep57 and Cep63 and the centriole targeting sites. They show that Cep57 is targeted to the centriole via its C-terminus and recruits Cep63 and then Cep152 in a hierarchical manner via its N-terminus. They then tested this in cells by generating Cep57 and Cep63 null U2OS cell lines. They show that Cep63 depletion impairs the recruitment of Cep152 but not of Cep57, as expected. However, the recruitment of Cep63 or Cep152 is affected by Cep57 depletion. The authors then searched for Cep57-related genes in the genomes of human and mouse and identified Cep57L1. Cep57L1 interacts with Cep63 via its N-terminus, like Cep57. Intriguingly, the same domain is required for centriole targeting, unlike Cep57 which localizes at centrioles via its C-terminus. A GFP-tagged version of Cep57L1 forms a ring around the proximal end of centrioles like Cep57, and behaves like Cep57 during mTEC differentiation. Zhao and colleagues then analyzed the effect of a

simultaneous depletion of Cep57 and Cep57L1 and found that it strongly affects the recruitment of Cep63 and Cep152, as well as centriole duplication. They propose that Cep57 and Cep57L1 cooperate to recruit Cep63 and Cep152 for centriole duplication.

Comments for the author

The comments I had made on the original version of the manuscript have been satisfactorily addressed in the revised version. I therefore support the publication of the work by Zhao and colleagues in the Journal of Cell Science.

I just have a comment on the terminology used to refer to centrioles, although there are differences of opinion on this point. It seems to me that, at least in G1, only the older centriole should be called mother centriole, the other being a daughter centriole. This is the terminology that many use, and in this sense the sentence " The Cep63-Cep152 complex located at the mother centriole recruits Plk4 to initiate centriole biogenesis" suggests that only the oldest centriole, i.e. the one with appendages, recruits Plk4. From S phase, the term mother centriole is used by some to design pre-existing centrioles, and daughter centriole for those that are assembling. I believe however that calling the latter procentrioles and the former centrioles or pre-existing centrioles is more accurate.

Reviewer 3

Advance summary and potential significance to field

The authors have done an excellent job with the revisions. This is an important advance, the work is of high quality, and I think it will be of high interest to the field.

Comments for the author

All requested revisions were addressed fully.

Second revision

Author response to reviewers' comments

Point-by-point responses:
Reviewer 1 Advance Summary and Potential Significance to Field:
This is a solid contribution describing the redundant functions of Cep57 and Cep57L1 in centriole biogenesis, and will be of interest to cell biologists working on centrioles.

Reviewer 1 Comments for the Author:
The authors have adequately addressed the concerns of the reviewers. My only remaining concern is that the authors need to carefully go over the figures and text to check for grammatical errors and English usage. As one example, in Figure 3B, the word mediate is used for intermediate, which is an unusual choice.

Response:
We thank the reviewer for appreciating our manuscript. We have asked an English native speaker to go through our manuscript to check for grammatical errors and English usage. In the revised manuscript, we have corrected these mistakes and highlighted them in red. Additionally, we have also changed “mediate” into “intermediate” in Fig. 3B according to the reviewer’s suggestion.

Reviewer 2 Advance Summary and Potential Significance to Field:
In this study, Zhao and colleagues analyzed the recruitment of Cep63 and Cep162 by Cep57 and a newly identified paralog of Cep57 called Cep57L1, during centriole assembly in U2OS and mTEC

cells. They first studied Cep57 localization during mTEC differentiation and showed that it is recruited at parental centrioles in a manner reminiscent of Cep63. Cep57 forms a ring around the proximal end of parental centrioles but is absent from deuterosomes. In later stages, Cep57 is recruited to the proximal end of all newly formed centrioles. The authors next used co-IP and GST-pull down assays to map the interaction domains between Cep57 and Cep63 and the centriole targeting sites. They show that Cep57 is targeted to the centriole via its C- terminus and recruits Cep63 and then Cep152 in a hierarchical manner via its N- terminus. They then tested this in cells by generating Cep57 and Cep63 null U2OS cell lines. They show that Cep63 depletion impairs the recruitment of Cep152 but not of Cep57, as expected. However, the recruitment of Cep63 or Cep152 is affected by Cep57 depletion. The authors then searched for Cep57-related genes in the genomes of human and mouse and identified Cep57L1. Cep57L1 interacts with Cep63 via its N-terminus, like Cep57. Intriguingly, the same domain is required for centriole targeting, unlike Cep57 which localizes at centrioles via its C-terminus. A GFP-tagged version of Cep57L1 forms a ring around the proximal end of centrioles like Cep57, and behaves like Cep57 during mTEC differentiation. Zhao and colleagues then analyzed the effect of a simultaneous depletion of Cep57 and Cep57L1 and found that it strongly affects the recruitment of Cep63 and Cep152, as well as centriole duplication. They propose that Cep57 and Cep57L1 cooperate to recruit Cep63 and Cep152 for centriole duplication.

Reviewer 2 Comments for the Author:
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Response:
We thank the reviewer for appreciating our work. To avoid the confusion on the usage of mother centrioles, we have added the definition of mother centrioles in the introduction section in the revised manuscript (Hatch et al., 2010).

Reviewer 3 Advance Summary and Potential Significance to Field:
The authors have done an excellent job with the revisions. This is an important advance, the work is of high quality, and I think it will be of high interest to the field.

Reviewer 3 Comments for the Author:
All requested revisions were addressed fully.

Response:
We thank the reviewer for appreciating our work.

Third decision letter

MS ID#: JOCES/2019/241836

MS TITLE: Cep57 and Cep57l1 function redundantly to recruit the Cep63-Cep152 complex for centriole biogenesis

AUTHORS: Huijie Zhao, Sen Yang, Xiaomeng Duan, Qingxia Chen, Guoqing Li, Qiongping Huang, Xueliang Zhu, and Xiumin Yan
ARTICLE TYPE: Short Report

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