Life Science Alliance

Centriole and transition zone structures in photoreceptor cilia revealed by cryoelectron tomography

Zhixian Zhang, Abigail Moye, Feng He, Muyuan Chen, Melina Agosto, and Theodore Wensel
DOI: https://doi.org/10.26508/lsa.202302409

Corresponding author(s): Theodore Wensel, Baylor College of Medicine

Review Timeline:

Submission Date:	2023-09-30
Editorial Decision:	2023-11-27
Revision Received:	2023-12-05
Editorial Decision:	2023-12-07
Revision Received:	2023-12-12
Accepted:	2023-12-12

Scientific Editor: Eric Sawey, PhD

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

November 27, 2023

Re: Life Science Alliance manuscript #LSA-2023-02409-T

Dr. Theodore G Wensel
Baylor College of Medicine
Biochemistry and Molecular Biology
One Baylor Plaza
Houston, Texas 77030-3411

Dear Dr. Wensel,

Thank you for submitting your manuscript entitled "Centriole and transition zone structures in photoreceptor cilia revealed by cryoelectron tomography" to Life Science Alliance. The manuscript was assessed by expert reviewers, whose comments are appended to this letter. We invite you to submit a revised manuscript addressing the Reviewer comments.

To upload the revised version of your manuscript, please log in to your account: https://lsa.msubmit.net/cgi-bin/main.plex

You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

While you are revising your manuscript, please also attend to the below editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

The typical timeframe for revisions is three months. Please note that papers are generally considered through only one revision cycle, so strong support from the referees on the revised version is needed for acceptance.

When submitting the revision, please include a letter addressing the reviewers' comments point by point.

We hope that the comments below will prove constructive as your work progresses.

Thank you for this interesting contribution to Life Science Alliance. We are looking forward to receiving your revised manuscript.

Sincerely,

Eric Sawey, PhD
Executive Editor
Life Science Alliance
http://www.lsajournal.org

A. THESE ITEMS ARE REQUIRED FOR REVISIONS

-- A letter addressing the reviewers' comments point by point.

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, https://www.life-science-alliance.org/authors

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title and running title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

-- By submitting a revision, you attest that you are aware of our payment policies found here: https://www.life-science-alliance.org/copyright-license-fee

B. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://www.life-science-alliance.org/authors

We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

IMPORTANT: It is Life Science Alliance policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images before submitting your revision.

Reviewer #1 (Comments to the Authors (Required)):

This is an excellent work describing the structural features of the base of the rod photoreceptor cilia. CryoET images of high quality show the arrangement of Y-shaped links and the ciliary necklace. The manuscript is well-written and was a pleasure to read. As described in the abstract, the structure obtained provides a scaffold for future studies that will identify proteins/molecules that are part of the cilia. The major claim is the clarity of the structural features provided, validation with TEM, twisting observed at the base, and organization of the ciliary necklace in rod photoreceptors. While the functional significance of the structures is unclear at this moment, the study provides a solid foundation upon which the field could build. One could argue that additional animal models are needed to validate the findings, but the approach is tedious and should be part of a separate study. Overall, the findings are of great interest to anyone interested in photoreceptor biology or ciliopathies.

Reviewer #3 (Comments to the Authors (Required)):

The submitted manuscript by Zhang and colleagues titled, "Centriole and transition zone structures in photoreceptor cilia revealed by cryo-electron tomography", provides the first in-depth cryoET analysis of the mouse rod photoreceptor cilium. They include analysis of the microtubule structure and associated protein densities found within the centrioles of the basal body and connecting cilium region of the axoneme. The authors discuss microtubule twisting, TMT to DMT transition, inner scaffold proteins, Y-links, and the ciliary necklace. This is the first study to analyze at the structure of the ciliary necklace. Their subtomogram averaging finds that the ciliary necklace is not bead-like but consists of a rectangular density that is composed of 5 ridges. This is a very interesting finding and the authors do well explaining how this structure is mis-represented single dense membrane particle in standard TEM images. Where this study falls short is in the shallowness of the cryoET data that prevents higher resolution structures to be resolved. It would have been a step above if the authors were able to localize or map known protein structures back onto the densities (even tubulin subunits). Without that information many of their conclusions feel vague. Words such as "unknown", "undetermined", "unclear" show up throughout the results when describing different densities or regions. One concern, is that despite this uncertainty, the authors are bold enough to state in the discussion that many of the current models need to be adjusted based on their data. Without identifying molecular complexes, I feel that the authors are overstating the power of their analysis. However, this study is the best structural analysis performed on photoreceptor cilia to date and is therefore an important advancement that should be considered for publication by Life Science Alliance. A list of major and minor remarks regarding the writing can be found below:

Major Remarks:
In the introduction the authors state "The region at the base of cilia where the microtubules undergo a transition from triplets to doublets is often referred to as the transition zone (TZ)". However, in the results the authors state "Note that the transition to doublets occurs well within the centrioles..." There is a disconnect between these two statements. If the transition to doublets is occurring within the mother centriole of the basal body, then why state that the TZ is marked by the transition from triplets to doublets? It is well described in the cilia field that the TZ is part of the axoneme structure. Even the citation the authors list in the introduction, Park and Leroux 2022, states that the "The first segment of the axoneme, termed transition zone (abbreviated TZ), contains typically Y-shaped structures, termed Y-links, that physically connect the doublet microtubules to the overlying ciliary membrane." The authors desire to attribute the TZ to the transition between triplets to doublets is unfounded, confusing, and should be removed.
Figure 6B lists n=6 and n=7 for the box and whisker plot. Does this n= measurements or cells or biological replicates?
Figure 6H does not provide n's for the plot.
In the first discussion section the authors start by saying the photoreceptor cilium is highly conserved, but also suggest current models should be adapted to include the differences they identified. It is not clear how the differences they observed would alter any of the current models. The authors need to discuss the major observed differences in detail, so this makes more sense.
In the second discussion section ends abruptly. Are the authors implying that the longitudinal twist of the MTs is providing structural integrity to the outer segment? If so, that needs to be clearly stated.

Minor Remarks:
Reference formatting error identified in the Introduction - References listed as (1,2) and (3) cannot be identified in the references as this list is alphabetical.

The use of abbreviations is not consistent throughout the manuscript. Microtubule (MT) is missing from the abbreviation list.
I am confused by the references listed as follows "high-pressure freezing and focused ion beam milling of more intact retina samples (Poge et al., 2021; Rigort et al., 2010; Young and Villa, 2023; Zhao et al., 2021)." These references did not perform high-pressure freezing and focused ion beam milling on intact retina samples.
Figure 3 Legend typo. Extra period should be removed "The twist angles β (defined in panel C) were measured and averaged for each cross-section and plotted in D for the cilium map and E for the centriole map. as a function of longitudinal position."

THEODORE G. WENSEL, PH.D.
Robert A. Welch Professor
Verna and Marrs McLean Department of Biochemistry and Molecular Pharmacology
One Baylor Plaza
MS: BCM125
Houston, TX 77030-3411
713-798-4528 office
713-796-9438 FAX

Eric Sawey, PhD
Executive Editor
Life Science Alliance
http://www.lsajournal.org

Dear Dr. Sawey,
Thank you for your message of Nov. 27, 2023 concerning our manuscript, "Centriole and transition zone structures in photoreceptor cilia revealed by cryoelectron tomography," by Zhixian Zhang et al. We have revised the manuscript in response to the reviewers' very useful and constructive comments. Our point-by-point response to those comments and the revisions made in response are listed below. The reviewers' comments are displayed in black Arial 11 point font and our responses are displayed in red 12 point Calibri font.
Reviewer #1 (Comments to the Authors (Required)):

This is an excellent work describing the structural features of the base of the rod photoreceptor cilia. CryoET images of high quality show the arrangement of Y-shaped links and the ciliary necklace. The manuscript is well-written and was a pleasure to read. As described in the abstract, the structure obtained provides a scaffold for future studies that will identify proteins/molecules that are part of the cilia. The major claim is the clarity of the structural features provided, validation with TEM, twisting observed at the base, and organization of the ciliary necklace in rod photoreceptors. While the functional significance of the structures is unclear at this moment, the study provides a solid foundation upon which the field could build. One could argue that additional animal models are needed to validate the findings, but the approach is tedious and should be part of a separate study. Overall, the findings are of great interest to anyone interested in photoreceptor biology or ciliopathies.
We thank the reviewer for their positive comments and agree that additional studies with animal models are needed. Such studies are currently ongoing in our laboratory but will take some time to complete.
Reviewer #3 (Comments to the Authors (Required)):

The submitted manuscript by Zhang and colleagues titled, "Centriole and transition zone structures in photoreceptor cilia revealed by cryo-electron tomography", provides the first in-depth cryoET analysis of the mouse rod photoreceptor cilium. They include analysis of the microtubule structure and associated protein densities found within the centrioles of the basal body and connecting cilium region of the axoneme. The authors discuss microtubule twisting, TMT to DMT transition, inner scaffold proteins, Y-links, and the ciliary necklace. This is the first study to analyze at the structure of the ciliary necklace. Their subtomogram averaging finds that the ciliary necklace is not bead-like but consists of a rectangular density that is composed of 5

ridges. This is a very interesting finding and the authors do well explaining how this structure is mis-represented single dense membrane particle in standard TEM images.
Where this study falls short is in the shallowness of the cryoET data that prevents higher resolution structures to be resolved. It would have been a step above if the authors were able to localize or map known protein structures back onto the densities (even tubulin subunits). Without that information many of their conclusions feel vague. Words such as "unknown", "undetermined", "unclear" show up throughout the results when describing different densities or regions. We agree that fitting known structures into our maps would have increased the information content; however, at our current resolution, while we could, for example, fit tubulin subunits into the maps, the resulting high-resolution pseudo maps would not be accurate, despite having the appearance of high resolution and molecular certainty. Performing such fits reliably will depend on improving further the resolution obtained *via* subtomogram averaging, and finding ways to identify unambiguously specific non-microtubule proteins.
One concern, is that despite this uncertainty, the authors are bold enough to state in the discussion that many of the current models need to be adjusted based on their data. Without identifying molecular complexes, I feel that the authors are overstating the power of their analysis. We have revised the discussion to avoid making such claims. The concluding sentence of the first paragraph of the discussion has been revised to read, “Some of these details suggest rods may differ in multiple ways from other mammalian sensory cilia, even in the regions proximal to the structurally highly divergent outer segment,” and does not mention existing models. However, this study is the best structural analysis performed on photoreceptor cilia to date and is therefore an important advancement that should be considered for publication by Life Science Alliance. A list of major and minor remarks regarding the writing can be found below:

Major Remarks:
In the introduction the authors state "The region at the base of cilia where the microtubules undergo a transition from triplets to doublets is often referred to as the transition zone (TZ)". However, in the results the authors state "Note that the transition to doublets occurs well within the centrioles..." There is a disconnect between these two statements. If the transition to doublets is occurring within the mother centriole of the basal body, then why state that the TZ is marked by the transition from triplets to doublets? It is well described in the cilia field that the TZ is part of the axoneme structure. Even the citation the authors list in the introduction, Park and Leroux 2022, states that the "The first segment of the axoneme, termed transition zone (abbreviated TZ), contains typically Y-shaped structures, termed Y-links, that physically connect the doublet microtubules to the overlying ciliary membrane." The authors desire to attribute the TZ to the transition between triplets to doublets is unfounded, confusing, and should be removed.
We have removed the text, “where the microtubules undergo a transition from triplets to doublets,” so the sentence now reads, "The region at the base of cilia is often referred to as the transition zone (TZ)".
Figure 6B lists n=6 and n=7 for the box and whisker plot. Does this n= measurements or cells or biological replicates? It is now made clear in the figure legend that n = cells
Figure 6H does not provide n's for the plot. These are now provided in the figure legend
In the first discussion section the authors start by saying the photoreceptor cilium is highly conserved, but also suggest current models should be adapted to include the differences they identified. It is not clear how the differences they observed would alter any of the current

models. The authors need to discuss the major observed differences in detail, so this makes more sense.
The text about adapting models has been removed to avoid confusion (see the revised text above).
In the second discussion section ends abruptly. Are the authors implying that the longitudinal twist of the MTs is providing structural integrity to the outer segment? If so, that needs to be clearly stated.
The following text has been added, “so a stabilization function for the twisting cannot be ruled out.”
Minor Remarks:
Reference formatting error identified in the Introduction - References listed as (1,2) and (3) cannot be identified in the references as this list is alphabetical. The references have been fixed.
The use of abbreviations is not consistent throughout the manuscript. Microtubule (MT) is missing from the abbreviation list. The use of abbreviations has been made consistent and “MT” as well as other abbreviations have been added to the list
I am confused by the references listed as follows "high-pressure freezing and focused ion beam milling of more intact retina samples (Poge et al., 2021; Rigort et al., 2010; Young and Villa, 2023; Zhao et al., 2021)." These references did not perform high-pressure freezing and focused ion beam milling on intact retina samples. The text has been altered to read, "high-pressure freezing and focused ion beam milling of more intact retina samples, as has been carried out for cryo-ET on other biological samples, including isolated rods (Pinskey et al., 2022; Poge et al., 2021; Rigort et al., 2010; Young and Villa, 2023)."

Figure 3 Legend typo. Extra period should be removed The extra period has been removed."The twist angles β (defined in panel C) were measured and averaged for each cross-section and plotted in D for the cilium map and E for the centriole map. as a function of longitudinal position."
[end of reviewers’ comments]
The revised manuscript and original figures have all been uploaded. We hope the revised paper will be found suitable for publication in Life Science Alliance.

Sincerely,

Theodore G. Wensel
Baylor College of Medicine
Welch Professor Department of Biochemistry and Molecular Pharmacology
Professor, Departments of Neuroscience and Ophthalmology
Director, Houston Area Molecular Biophysics Program

December 7, 2023

RE: Life Science Alliance Manuscript #LSA-2023-02409-TR

Dr. Theodore G Wensel
Baylor College of Medicine
Biochemistry and Molecular Pharmacology
One Baylor Plaza
Houston, Texas 77030-3411

Dear Dr. Wensel,

Thank you for submitting your revised manuscript entitled "Centriole and transition zone structures in photoreceptor cilia revealed by cryoelectron tomography". We would be happy to publish your paper in Life Science Alliance pending final revisions necessary to meet our formatting guidelines.

Along with points mentioned below, please tend to the following:
-please add your main, supplementary figure, and table legends to the main manuscript text after the references section
-please add a conflict of interest statement to your main manuscript text
-please use the [10 author names et al.] format in your references (i.e., limit the author names to the first 10)
-there are call outs for figures S6, S7A and these figures are not provided -- please correct
-please add callouts for Figures 7C; S1A-B; S3A-C; S4A-C; S5A-E to your main manuscript text

Figure Checks:
-In Figure 6C, the resolution of the bottom right panel appears less than the other panels. If this can be improved, please do so.

If you are planning a press release on your work, please inform us immediately to allow informing our production team and scheduling a release date.

LSA now encourages authors to provide a 30-60 second video where the study is briefly explained. We will use these videos on social media to promote the published paper and the presenting author (for examples, see https://twitter.com/LSAjournal/timelines/1437405065917124608). Corresponding or first-authors are welcome to submit the video. Please submit only one video per manuscript. The video can be emailed to contact@life-science-alliance.org

To upload the final version of your manuscript, please log in to your account: https://lsa.msubmit.net/cgi-bin/main.plex
You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. FINAL FILES:

These items are required for acceptance.

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, https://www.life-science-alliance.org/authors

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

B. MANUSCRIPT ORGANIZATION AND FORMATTING:

Full guidelines are available on our Instructions for Authors page, https://www.life-science-alliance.org/authors

We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

Submission of a paper that does not conform to Life Science Alliance guidelines will delay the acceptance of your manuscript.

It is Life Science Alliance policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.

The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be available to the corresponding author only. Please take a moment to check your funder requirements.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days.

Thank you for this interesting contribution, we look forward to publishing your paper in Life Science Alliance.

Sincerely,

Eric Sawey, PhD
Executive Editor
Life Science Alliance
http://www.lsajournal.org

December 12, 2023

RE: Life Science Alliance Manuscript #LSA-2023-02409-TRR

Dr. Theodore G Wensel
Baylor College of Medicine
Biochemistry and Molecular Pharmacology
One Baylor Plaza
Houston, Texas 77030-3411

Dear Dr. Wensel,

Thank you for submitting your Research Article entitled "Centriole and transition zone structures in photoreceptor cilia revealed by cryoelectron tomography". It is a pleasure to let you know that your manuscript is now accepted for publication in Life Science Alliance. Congratulations on this interesting work.

The final published version of your manuscript will be deposited by us to PubMed Central upon online publication.

Your manuscript will now progress through copyediting and proofing. It is journal policy that authors provide original data upon request.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

IMPORTANT: If you will be unreachable at any time, please provide us with the email address of an alternate author. Failure to respond to routine queries may lead to unavoidable delays in publication.

Scheduling details will be available from our production department. You will receive proofs shortly before the publication date. Only essential corrections can be made at the proof stage so if there are any minor final changes you wish to make to the manuscript, please let the journal office know now.

DISTRIBUTION OF MATERIALS:
Authors are required to distribute freely any materials used in experiments published in Life Science Alliance. Authors are encouraged to deposit materials used in their studies to the appropriate repositories for distribution to researchers.

You can contact the journal office with any questions, contact@life-science-alliance.org

Again, congratulations on a very nice paper. I hope you found the review process to be constructive and are pleased with how the manuscript was handled editorially. We look forward to future exciting submissions from your lab.

Sincerely,

Eric Sawey, PhD
Executive Editor
Life Science Alliance
http://www.lsajournal.org