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Three Dimensional Structure of Basal Body Triplet Revealed by Electron Cryo-Tomography

Sam Li, Jose-Jesus Fernandez, Wallace F Marshall and David A Agard

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(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.)

1st Editorial Decision 15 September 2011

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are enclosed. As you will see, all three referees express significant interest in your work, and are broadly in favour of publication, pending satisfactory revision. Their reports are explicit, so I need not go into detail here, although I would in particular draw your attention to the comments of referee 2 re. the need to better discuss and cite relevant literature.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of all three reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version. When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
http://www.nature.com/emboj/about/process.html

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1

The manuscript "Three Dimensional Structure of Basal Body Triplet Revealed by Electron Cryo-Tomography" by Sam Li et al addresses the structure of the basal body using cryo tomography.

The authors report the structure of the basal body (bb) triplet at 33 Å resolution and by comparing it with the atomic structure of tubulin, they identify many new densities along the structure that are likely to represent novel protein complexes that stabilize and regulate the bb. Additionally they find structural variations along the structure from proximal to distal.

In general I think this is a really nice manuscript that will pave the way to identify and fit in the different complexes that assemble the structure.

I have some concerns and suggestions:

Concerns:
1-Perhaps the authors should justify better why they do not see the cartwheel- is this common in basal body isolation from Chlamy? Or could this reflect problems/defects in the isolated structures?

2-The authors propose a model in which δ- tubulin participates in the assembly of the basal body- in their discussion they claim that:
*they see a density in the proximal part of the basal body- C1- that cannot be fitted by tubulin.
*since mutants in δ- tubulin show problems in forming the C tubule and this can be rescued by mutations in tubulin (Fromherz et al, 2004), perhaps δ- tubulin is C1
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The last argument is not correct. Drosophila shows basal bodies with triplets in the sperm (eg. See any paper from Giuliano Callaini or the review from Azimzadeh et al Curr Biol 2010 for a discussion on exactly this subject, or Gonzalez et al, JCS, 1998 for a review)

This discussion should be reformulated if they want to provide any mechanistic insight. Is the mutant tubulin that rescues the δ-tubulin mutant (Fromherz et al, 2004) more similar in structure to δ-tubulin? Is the Drosophila tubulin more similar to δ-tubulin (Drosophila shows some specific tubulin isoforms)? Is the C1 density missing in the delta tubulin mutants?

Suggestions:

1-can the authors propose a 3D model of the whole structure (minus the cartwheel that they do not see)- would be very nice for the community (in Figure 6 and in an animation in the web).

2-Supplementary Figure 3 should be shown in Figure 5 to illustrate how they calculate the angles

3-Fig 1a please explain what you call probasal body

4-page 8, explain what is Z-rise

5-justify why they think densities observed in Figure 2B and C are tektin

6-would be nice to show data from the triplet to doublet transition- how does the C-tubule end- can the authors see this transition?

7-perhaps it would be nice to have a table summarizing the new structures observed and predicted sizes to really pave the road for their identification

Referee #2

This manuscript reports the first (to my knowledge) cryo-EM study of basal bodies by a leading group in the use of this technology. The study uses advanced imaging, image processing and modeling techniques to reveal a new level of structural detail about the microtubule triplets in basal bodies and reveals previously uncharacterized associated structures. While the study may be considered "descriptive" of the structure, the level of resolution of intriguing densities and linkages in the triplet microtubules is impressive. It is possible that this study will be viewed as a seminal basal body paper and will be the basis for future analysis of basal bodies in other cell types, for the localization of components and for the analysis of mutant basal bodies.

My significant enthusiasm for this work is dampened by some shortcomings in manuscript. The authors fail to put their structural work in context of previously completed work, but use space in the discussion for highly speculative roles of delta-tubulin and POC5 in basal body assembly. For instance, cryo-EM of centrioles has been reported (Guichard, Chrétien, Marco, and Tassin (2010) Procentriole assembly revealed by cryo-electron tomography EMBO J 29:1565-1572.), but the paper is not even cited. It would be surprising if there are no worthwhile similarities or differences between these centriole and basal body cryo-EM studies to point out in the discussion. Similarly, there is a significant literature on Chlamydomonas basal body structure from in situ electron tomography work, yet this work is not discussed in relation to the new cryo-EM structures presented in this manuscript. For instance, is there in vivo data for the structures on luminal side of the basal body microtubule triplets? Such lapses are also the basis of the numerous minor issues listed below.

Minor issues:

1. The list of classic studies on basal body structure and assembly at the top of page 4 is welcome, and should include:

Allen (1969) The morphogenesis of basal bodies and accessory structures of the cortex of the ciliated protozoan Tetrahymena pyriformis. J Cell Biol. 40:716-733.

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2. In the following paragraph, many studies using proteomics and genomics to identify centriole/basal body components have been reported. There needs to be a more complete description of this body of work.

3. In the Introduction (page 4) the purified basal bodies are said to be in a "native state," but at the beginning of the Results section the claim is "near-native state." What does this mean? The cartwheel structure is absent - it's hard to claim the "native state." They are enriched basal bodies that retain the canonical microtubule triplets and some associated structures.

4. In the Results section, a comment is made about the A/C linkages being significantly different from proximal to distal. Looking at Fig. 5 A and B, each linkage (4 shown, 2 proximal, 2 distal) looks slightly different but related, even from 1 triplet to the next in the same region. Calling these linkages significantly different seems strong, or needs to be more clearly explained.

5. It would be helpful to have the thickness in nm of the individual and combined subvolumes (distal and proximal) listed in the figure legend of 4A. There is a description in the Material & Methods, but is it the description is for all of the averaging or just the original averaging of the triplet structure?

6. The right panel of Fig. 4A maybe made clearer by showing similar views of A tubule protofilaments. Similarly, some arrows indicating the change in periodicity of C1 from 4nm in the proximal end to 8nm in the distal end would be helpful in Fig. 4B. How close to the end of the C

tubule is the distal end data?

7. Are there variations between the triplets within the basal body? Is the data for the averaged triplets from triplets in the same position in each basal body, or from different triplets in the basal body that is being sampled?

8. At the beginning of the Discussion at the bottom of page 16 and onto 17 suggests that the less elaborate features of the A and B tubule possibly reflect less stress on the structure than that found in axenomes. It is worth mentioning that that A and B tubules may also be bolstered by the C tubule, hence changing the requirements for modifications of these tubules.

Referee #3

In this paper the authors provide a description of the structure of Chlamydomonas basal body microtubule triplets obtained by cryo-electron tomography. The work is mainly descriptive, rather than providing any great functional insight, but the level of detail in the description is remarkably beyond what has been available up to now. The resolution obtained by working with frozen-hydrated material and applying subvolume averaging reveals a wealth of new detail that includes a number of surprises and a great deal of insight. Variations of elements that have generally been assumed to be very static are particularly notable. Non-tubulin components that provide various links between the A, b and C tubules as well as between the triplets themselves are very nicely defined. Identifying the proteins that make up these links is obviously the next step, and this paper defines the nature of what needs to be done in an uncommonly clear way. The authors do speculate on the possible locations and roles of two known components of the basal body, delta tubulin and POC5. While the evidence that they provide for identification of these proteins is not overwhelming, the discussion gives an idea of the types of questions that can now be addressed. In short, the results provide a very provocative new look at a structure that has both intrigued scientists for decades and recently been recognized for its role in a number of diseases. Thus I expect that this work will inspire a significant wave of further investigations.

The paper is quite nicely organized and written. My only suggestion relates to page 13 where they talk about connections to one or another tubulin helix. It took me a while to realize that they were talking about the 3-start helices of the microtubule structure, and some reference to this structural feature would certainly help some readers.

1st Revision - authors' response 02 November 2011

Referee #1

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In general I think this is a really nice manuscript that will pave the way to identify and fit in the different complexes that assemble the structure.

I have some concerns and suggestions:

Concerns:
1-Perhaps the authors should justify better why they do not see the cartwheel- is this common in basal body isolation from Chlamy? Or could this reflect problems/defects in the isolated structures?

Response: Unlike the human centriole where the cartwheel is lost after the maturation, in Chlamy, the cartwheel remains in the basal body throughout the life cycle. However, using the same well-

established isolation procedure used for Chlamy proteomics studies, we did not observe the cartwheel in our tomogram. It is likely that most cartwheel components have disassociated from the basal body during the purification process, even though the triplets in the probasal body remain (as shown in Fig1A in manuscript). This loss is consistent with the basal body proteomic study, in which most of cartwheel components, such as the Bld12p (homolog of Sas-6) that forms the hub, are undetectable. However, Bld10p (homolog of Cep135) at the tip of the spoke responsible for nucleating the triplet microtubule remains in the basal body proteome. Likely it is also present in our tomograms. Even though this segment of the basal body is not our main focus in this study, in the future, it will be interesting to average the probasal body triplet to see if there are any structural differences compared to our current triplet structure. Meanwhile, we do need to optimize the basal body purification procedure to preserve the cartwheel structure.

2-The authors propose a model in which delta-tubulin participates in the assembly of the basal body- in their discussion they claim that:
**they see a density in the proximal part of the basal body- C1- that cannot be fitted by tubulin.*
**since mutants in delta-tubulin show problems in forming the C tubule and this can be rescued by mutations in tubulin (Fromherz et al, 2004), perhaps delta-tubulin is C1*
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The last argument is not correct. Drosophila shows basal bodies with triplets in the sperm (eg. See any paper from Giuliano Callaini or the review from Azimzadeh et al Curr Biol 2010 for a discussion on exactly this subject, or Gonzalez et al, JCS, 1998 for a review)

Response: It is indeed the case that in Drosophila sperm the basal body contains triplets instead of doublets or singlets. We have now stated in the text (p.21) "…Interestingly, both δ-tubulin and POC5 genes are absent in organisms such as Caenorhabditis elegans and Drosophila melanogaster, where only singlets or doublets are observed in the centriole or basal body in the somatic cells (Azimzadeh et al, 2009; Dutcher, 2001), further suggesting their unique roles in assembly of the C-tubule of the triplet. However, triplets have been observed in the basal bodies and centrioles in germ cells in Drosophila (Mahowald & Strassheim, 1970; Riparbelli & Callaini, 2011). It will be interesting to investigate the tissue specific expression levels of different tubulin isoforms, as this might provide insight into their specific roles during the basal body/centriole assembly."

This discussion should be reformulated if they want to provide any mechanistic insight. Is the mutant tubulin that rescues the delta-tubulin mutant (Fromherz et al, 2004) more similar in structure to delta-tubulin? Is the Drosophila tubulin more similar to delta-tubulin (Drosophila shows some specific tubulin isoforms)? Is the C1 density missing in the delta-tubulin mutants?

Response: Chlamy has two identical copies of alpha-tubulin genes. The mutant TUA2, which partially suppresses the delta-tubulin deletion (UNI3) phenotype, has two mutation sites, D205N and A208T (Fromherz et al 2004). The mutations are localized on the helix6 in the tubulin structure that is postulated to be involved in the plus-end longitudinal interactions between two neighboring tubulins along the protofilament. This indicates that the suppressor mutant TUA2 might have an altered tubulin-tubulin affinity, which is able to partially compensate for the loss of delta-tubulin. However, the molecular mechanism of this suppression is unknown. This suppression could be either direct or indirect, as Fromherz et al have proposed (Fromherz, et al 2004). Neither of the mutations (D205N and A208T) makes alpha-tubulin more like delta-tubulin, which has 205E and 208A at the equivalent positions. Since another copy of the alpha-tubulin gene TUA1 is tightly linked to UNI3 on the linkage group, it is possible that the deletion of UNI3 gene in uni3-1 mutant made the TUA1 gene less accessible to mutagenesis. Alternatively, slightly different expression patterns between TUA1 and TUA2 seem to have made TUA2 a more favorable site of mutation for suppression.

In Chlamy, the C-tubule is absent in the proximal half of the basal body in the delta-tubulin deletion mutant. Although a previous EM tomography study of the basal body from the Chlamy cell could not pinpoint whether or not the C1 density is missing in the mutant, we speculate this is the case since the C1 is at a unique position that initiates the branching of the C-tubule from the B-tubule. Experiments are now underway to study the basal body from delta-tubulin deletion mutant uni3-1. We hope these will shed light on the role of the delta-tubulin on the basal body assembly.

The Drosophila genome contains 4 genes encoding alpha-tubulin isoform 1 to 4. The first 3 isoforms are almost identical with pairwise identity about 95%. Isoform 4 has 68% protein sequence identity with the other three isoforms. However, all have low sequence identity with human delta-tubulin (near 24%). Therefore, it is not clear if a particular isoform of alpha-tubulin is responsible for the triplet formation in the sperm basal body. In the future, it will be very interesting to investigate the tissue specific distribution of tubulin isoforms in Drosophila, such as in the Drosophila sperm, and to find out if any particular isoform of tubulin might replace delta-tubulin in making the basal body triplet.

Suggestions:

1-can the authors propose a 3D model of the whole structure (minus the cartwheel that they do not see)- would be very nice for the community (in Figure 6 and in an animation in the web).
Response: In "Building a basal body model" subsection in the Results section (p.14), we have described in detail our basal body model based on the averaged triplet structure. The models are illustrated in Fig 5 as the proximal and distal end respectively. Meanwhile, by interpolating the structures between the proximal and the distal ends, we now have generated an ideal model for the entire basal body (albeit lacking the cartwheel) and have created an animation that is available on our lab website (http://www.msg.ucsf.edu/agard/people/Li/sam.html).

2-Supplementary Figure 3 should be shown in Figure 5 to illustrate how they calculate the angles
Response: we have now merged the Fig 3S into Figure 5 to show how the triplet rotation angles are defined and calculated.

3-Fig 1a please explain what you call probasal body
Response: We followed the probasal body (abbr. pbb) convention used previously by others (Geimer & Melkonian, 2004; O'Toole, et al., 2003; Piasecki, et al., 2008). The probasal body is defined as the proximal segment of the basal body about 100 nm in length where multiple tiers of cartwheels are localized at the early stage of basal body duplication. This is also the segment before the emergence of "the A-tubule feet" as shown in Fig 1a. We have now clarified this in the figure legend of Fig1a.

4-page 8, explain what is Z-rise
Response: We have now modified the text as "The z-rise (longitudinal rise) of tubulins between adjacent PFs, varies from 10 to 12 Å".

5-justify why they think densities observed in Figure 2B and C are tektin
Response: The localization and the possible functions of tektins are inferred from previous studies, especially a recent cryo-tomography reconstruction study of the axonemal doublet microtubule (Sui and Downing, 2006), which revealed the tektins are most likely in the lumen of the tubules that cross-link the microtubule protofilaments in the axonemal doublets. Since tekins are major structural components in both axoneme and basal body, it is likely tektins have similar location and function in both structures. On p.10 of the text, we have cited a recently comprehensive review on tektins by L.A. Amos (Amos, 2008)

6-would be nice to show data from the triplet to doublet transition- how does the C-tubule end- can the authors see this transition?
Response: Indeed, it will be very interesting to see the transition of triplet to doublet and to understand the termination mechanism of the C-tubule in triplet. Experiments are now underway to reveal the structure of this transition and also to identify components playing roles in the C-tubule termination. To obtain comparable quality results, we will need to average data from many more basal bodies.

7-perhaps it would be nice to have a table summarizing the new structures observed and predicted sizes to really pave the road for their identification
Response: In this study, we first found a repetitive Y-shaped structure that cross-links the A and B-tubules in the luminal side of the basal body with an estimated mass of 1.1 MDa. Secondly, in the distal half of the basal body, we saw a hook-shaped structure with estimated mass of 230 KDa at the C1 position. Finally, there is a filamentous structure in the lumen of the C-tubule with estimated

mass of 46 KDa. While we completely agree with the reviewer's goals, because of the limitation of current resolution at 33 angstrom, it is likely the new structures observed are multi-protein complexes, instead of individual components. This makes identification much more difficult. Although it will be a good idea to systematically analyze the new densities, including their shapes and predicted sizes and masses, we think it is equally important to find the molecular identity of these complexes, and to improve the resolution so that most individual molecules or domain can be resolved. In this way, with both the theoretical and experimentally estimated masses of the known basal body components in hands, it will facilitate the identification of their locations in context of 3D structure of the basal body.

Referee #2

This manuscript reports the first (to my knowledge) cryo-EM study of basal bodies by a leading group in the use of this technology. The study uses advanced imaging, image processing and modeling techniques to reveal a new level of structural detail about the microtubule triplets in basal bodies and reveals previously uncharacterized associated structures. While the study may be considered "descriptive" of the structure, the level of resolution of intriguing densities and linkages in the triplet microtubules is impressive. It is possible that this study will be viewed as a seminal basal body paper and will be the basis for future analysis of basal bodies in other cell types, for the localization of components and for the analysis of mutant basal bodies.

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Response: we do apologize for previously not citing the work of Guichard et al. on the study of the procentriole by EM cryo-tomography. We have now included this reference and cited it on p.4 of the main text. By using cryo-electron tomography, the work done by Guichard et al. has provided new insight into the assembly mechanism of the human procentriole, especially the sequential assembly of the A, B and C-tubules and the role of gamma-TuRC during the triplet formation have now been revealed in unprecedented details. Although the work is extremely interesting, it is mainly focused on the kinetics of the procentriole formation, equivalent to the probasal body section in the basal body. In our work, we are focused on the structural analysis of the basal body, which is an extension of the probasal body. In our purified basal bodies, we have not been able to capture the intermediate stages of the A-, B- and C-tubule formation. Therefore, even though these two works are highly complementary, we did not elaborate in great detail the comparison of these two works in our manuscript.

There have been numerous previous publications on the study of Chlamy basal body structure, either by conventional serial section electron microscopy or electron tomography, for example, works by Cavalier-Smith, by Ringo, by Eileen O'Toole et al. and by Stephan Geimer et al. We now make our references more comprehensive. For example, on p.18-19 in discussion of the structure on luminal side of the basal body triplet, we have cited works previous observations by Cavalier-Smith, Geimer & Melkonian, Ibrahim et al, Paintrand et al, Fais et al, Piasecki et al and Vorobjev & Chentsov.

Minor issues:

1. The list of classic studies on basal body structure and assembly at the top of page 4 is welcome, and should include:

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ciliated protozoan Tetrahymena pyriformis. J Cell Biol. 40:716-733.

Anderson and Brenner (1971) The formation of basal bodies (centrioles) in the Rhesus monkey oviduct. Cell Biol. 50:10-34.

In fact, the Allen study should probably replace the Giddings citation.

Response: We have now included the above two papers on p.4 as references.

2. In the following paragraph, many studies using proteomics and genomics to identify centriole/basal body components have been reported. There needs to be a more complete description of this body of work.
Response: We have now added the following references on p.4:

Kilburn CL, Pearson CG, Romijn EP, Meehl JB, Giddings TH, Culver BP, Yates JR, Winey M (2007) New Tetrahymena basal body protein components identify basal body domain structure. J Cell Biol **178**(6)**:** 905-912

Li JB, Gerdes JM, Haycraft CJ, Fan Y, Teslovich TM, May-Simera H, Li H, Blacque OE, Li L, Leitch CC, Lewis RA, Green JS, Parfrey PS, Leroux MR, Davidson WS, Beales PL, Guay-Woodford LM, Yoder BK, Stormo GD, Katsanis N, Dutcher SK (2004) Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. Cell **117**(4)**:** 541-552

3. In the Introduction (page 4) the purified basal bodies are said to be in a "native state," but at the beginning of the Results section the claim is "near-native state." What does this mean? The cartwheel structure is absent - it's hard to claim the "native state." They are enriched basal bodies that retain the canonical microtubule triplets and some associated structures.
Response: We were trying to be conservative by saying near native state. Here, the term "near-native state" has a two-fold meaning. First, during purification steps, the basal bodies were extracted under near-physiological conditions with neutral pH and low-salt buffer. The cell membrane was extracted by low percentage of mild detergent NP-40. The number of purification steps was limited to minimize the perturbation of basal body structure, although the cartwheel structures are lost by the low salt treatment. Second, throughout the entire EM tomography data acquisition process, the basal body sample was kept under liquid nitrogen conditions (-180ºC), and the accumulative electron dose was kept below 80e^-/Å^2, so that the electron radiation damage was minimized. With all these efforts, we believe the basal body sample was visualized in "near-native state" in this study.

To be consistent, we have now changed text to "near-native state".

4. In the Results section, a comment is made about the A/C linkages being significantly different from proximal to distal. Looking at Fig. 5 A and B, each linkage (4 shown, 2 proximal, 2 distal) looks slightly different but related, even from 1 triplet to the next in the same region. Calling these linkages significantly different seems strong, or needs to be more clearly explained.

Response: On p.14 and in Fig 5A and B, we indicated that the A/C linkage between adjacent triplets is significantly different from the proximal to the distal end of the basal body. This different is evident with the 10-degree rotation of triplets from the proximal to the distal end, which is shown in Fig 5C. Now we indicate this difference more explicitly by adding arrows in the figures (Fig. 5A and B) and by stating on p.14-15 "… a comparison of the two ends reveals that the inter-triplet linkages are significantly different (black arrows in the right panel of Fig. 5A and B respectively)."

5. It would be helpful to have the thickness in nm of the individual and combined subvolumes (distal and proximal) listed in the figure legend of 4A. There is a description in the Material & Methods, but is it the description is for all of the averaging or just the original averaging of the triplet structure?
Response: Now in the figure legend for Fig. 4A, we state: "Each group contains a triplet segment about 39 nm in length." In the figure legend for Fig 4B and C, we note "The longitudinal length of the triplets in Fig. 5B and C is about 26 nm". The detail of how these structures have been derived is described in the "Materials and Methods" section of the manuscript.

6. The right panel of Fig. 4A maybe made clearer by showing similar views of A tubule protofilaments. Similarly, some arrows indicating the change in periodicity of C1 from 4nm in the proximal end to 8nm in the distal end would be helpful in Fig. 4B. How close to the end of the C tubule is the distal end data?
Response: Now we have added arrows in Fig. 4A to indicate the change. The distal end data is about 45 nm away from the end of C-tubule as we have stated on p.6 and indicated in Fig. 1A.

7. Are there variations between the triplets within the basal body? Is the data for the averaged triplets from triplets in the same position in each basal body, or from different triplets in the basal body that is being sampled?
Response: The structure is from the average over all the triplets in the basal bodies that are being sampled. We also have investigated to see if there is any variation among the triplets within the basal body, also known as rotational polarity or lateral asymmetry of the basal body. First, following the Hoops & Witman numbering convention of the triplets (JCB, 1983), we classified the triplets from basal bodies into 9 groups according to their positions in the basal body and by using the associated distal striated fibers as a reference point. Then, we averaged subvolumes from each group of triplets (total 216 subvolumes in each group). This resulted in 9 averaged triplet structures. We compared these averaged structures. At current resolution (30~40 Å), we did not observe significant differences. There might be several reasons for this,

A. The current number of subvolumes in each group for averaging (216 subvolumes per group) is limited. The difference in the averaged structure from each group is not significant enough at current resolution.
B. Instead of being along the entire triplet length, the asymmetry is only localized to certain longitudinal position of the triplet, therefore after averaging, the different (asymmetric) structures have been "averaged out" and obscured. In the future, this problem can be overcome by increasing the pool of data, and by averaging subvolumes from only certain groups of triplets at certain longitudinal positions. This is something we would like to do.

8. At the beginning of the Discussion at the bottom of page 16 and onto 17 suggests that the less elaborate features of the A and B tubule possibly reflect less stress on the structure than that found in axenomes. It is worth mentioning that that A and B tubules may also be bolstered by the C tubule, hence changing the requirements for modifications of these tubules.
Response: We now have modified the text on p.18 as "likely because the basal body bears less mechanical stress from its cellular environment and the A- and B-tubule are further bolstered by the C-tubule.

Referee #3

In this paper the authors provide a description of the structure of Chlamydomonas basal body microtubule triplets obtained by cryo-electron tomography. The work is mainly descriptive, rather than providing any great functional insight, but the level of detail in the description is remarkably beyond what has been available up to now. The resolution obtained by working with frozen-hydrated material and applying subvolume averaging reveals a wealth of new detail that includes a number of surprises and a great deal of insight. Variations of elements that have generally been assumed to be very static are particularly notable. Non-tubulin components that provide various links between the A, b and C tubules as well as between the triplets themselves are very nicely defined. Identifying the proteins that make up these links is obviously the next step, and this paper defines the nature of what needs to be done in an uncommonly clear way. The authors do speculate on the possible locations and roles of two known components of the basal body, delta tubulin and POC5. While the evidence that they provide for identification of these proteins is not overwhelming, the discussion gives an idea of the types of questions that can now be addressed. In short, the results provide a very provocative new look at a structure that has both intrigued scientists for decades and recently been recognized for its role in a number of diseases. Thus I expect that this work will inspire a significant wave of further investigations.

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feature would certainly help some readers.
Response: We have now modified text on p.13 as
"Assuming the triplet C-tubule has the microtubule B-surface lattice and can be seen as a bundle of tubulin helices (red arrows in Fig. 4C) (Chretien D & Wade R, 1991; Song YH & Mandelkow E, 1995), the tilting of the filament is in the opposite direction to the tilting of the tubulin helices, indicating that the luminal filament most likely attaches to one tubulin helix at the C3 end and attaches to the adjacent tubulin helix running underneath at the C6/C7 end. These attachments suggest that the filament has intrinsic polarity with two ends making unique contacts with the C-tubule."

Additional Correspondence 15 November 2011

Thank you for your help with manuscript EMBOJ-2011-79154R, "Three Dimensional Structure of Basal Body Triplet Revealed by Electron Cryo-Tomography". For your records, the editor's decision for this manuscript, based partly on your input, was Accept.

Your assistance and participation in the review process for The EMBO Journal is greatly appreciated.

Please see other reviewers comments for this manuscript at the bottom of the page.

Sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #2

The authors have done a very careful and thorough revision addressing all of my previous conerns, as well as the concerns from the other reviewers. This manuscript is in excellent shape. I am excited to see this cryo-EM study of basal bodies published and to see its influence on the structural analysis of basal bodies and centrioles.

