Figures and figure supplements

Electron cryo-tomography provides insight into procentriole architecture and assembly mechanism

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Figure 1. Tomographic Reconstruction and Subtomogram Averaging of the MT Triplet in Centriole and Procentriole. (A) A slice from the reconstructed tomogram showing a pair of mother centrioles, also known as basal bodies. The central hub as part of the cartwheel is marked by a yellow asterisk. The centriole is partitioned longitudinally into three regions. The procentriole region spans 100 nm at the proximal end of the mother centriole. The central core region spans 250 nm. The distal region spans 150 nm where the triplets become doublets before reaching the transition zone (TZ). PSF: proximal striated fibers; DSF: distal striated fibers; rMT: rootlet MT. (B) A slice from the reconstructed tomogram of procentriole attached to the mother centriole via the rootlet MT. The central hub, radial spokes and pinheads are clearly visible, as well as protofilaments in some MT triplets. RS: radial spoke; PH: pinhead; rMT: rootlet MT. (C) Subtomogram average of the MT triplet from both the proximal region of mother centrioles and the procentrioles. (D) A Figure 1 continued on next page
model of procentriole viewed from its distal end. The model is generated by docking the subtomograms average in (C) into the tomogram volume in (B). The A- B- and C-tubules and the A-C linker are labeled. The central hub and the radial spokes are depicted schematically. PH: pinhead.

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Figure 1—figure supplement 1. Assessing the Resolutions of Subtomogram Average by Fourier Shell Correlation (FSC) Method. FSC curves are for the MT triplet (black, 23.0 Å), the A-tubule (red, 21.4 Å), the B-tubule (blue, 22.2 Å) and the C-tubule (green, 22.2 Å). All resolution assessments are carried out in a ‘gold-standard’ Scheme using FSC 0.143 criterion.

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Figure 1—figure supplement 2. Comparing Subtomogram Averages from a Subset of Data. (A) The triplet in blue on left is an average based on 10854 subtomograms from the proximal end of mother centrioles. (B) The triplet in light green on right is an average based on 2083 subtomograms from the 110 procentrioles that remain attached to the mother centrioles. The differences between these two structures are indicated by red arrows and by red asterisks, respectively.

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Figure 2. Identifying Non-tubulin Components in Procentriole MT Triplet. (A) The A-tubule structure. A model of 13-pf MT (red) are docked into the subtomogram average of the A-tubule. 4 MIPs identified on the A-tubule are highlighted in colors. (B) The B-tubule structure. A model of 10-pf MT
Figure 2 continued

(blue) are docked into the subtomogram average of the B-tubule. 4 MIPs identified on the B-tubule are highlighted in colors. (C) The C-tubule structure. A model of 10-pf MT (green) are docked into the subtomogram average of the C-tubule. 3 MIPs found at the B-C inner junction are highlighted in colors. (D) Subtomogram averaging of the pinhead structure. The pinhead is highlighted in pink color. Two dashed arrows indicate viewing directions for two images in the side view on the right. The arrows indicate PinB, PinF1 and PinF2, respectively. The arrowheads mark the tip of PinB.

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**Figure 2—figure supplement 1.** The non-tubulin Procentriole Components Associated with the MT Triplet. The figures show the binding pattern of 11 MIPs and their periodicity along the MT wall. In each figure, image on the left is a longitudinal cross section of the average of corresponding tubule. The middle image is a surface-rendered map with the structure of corresponding MIP highlighted in color. Image on the right is a cartoon model of the tubule. The dashed line indicates the location where the cross section goes through. The red arrow indicates viewing direction of the structure shown in the middle image. They are also summarized in Table 1.

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Figure 2—figure supplement 2. The non-tubulin Procentriole Components Associated with the MT Triplet.
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Figure 2—figure supplement 3. The non-tubulin Procentriole Components Associated with the MT triplet.
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Figure 2—figure supplement 4. The non-tubulin Procentriole Components Associated with the MT Triplet.
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Figure 2—figure supplement 5. The non-tubulin Procentriole Components Associated with the MT Triplet.
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Figure 2—figure supplement 6. The non-tubulin Procentriole Components Associated with the MT Triplet.
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Figure 2—figure supplement 7. The non-tubulin Procentriole Components Associated with the MT Triplet.
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Figure 2—figure supplement 8. The non-tubulin Procentriole Components Associated with the MT Triplet.
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**Figure 2—figure supplement 9.** The non-tubulin Procentriole Components Associated with the MT Triplet.
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Figure 2—figure supplement 10. The non-tubulin Procentriole Components Associated with the MT Triplet.
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Figure 2—figure supplement 11. The non-tubulin Procentriole Components Associated with the MT Triplet.
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Figure 2—figure supplement 12. MIP9 forms a non-tubulin filament at the inner junction of B- and C-tubule. The MIP9 density is highlighted in blue. A model of MT protofilament in green is fit into the MIP9 density. On the right panel, only the MIP9 filament is shown in two orthogonal views, the rest triplet structure is removed for clarity. The MIP9 density differs substantially from a canonical MT protofilament.

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Figure 2—figure supplement 13. FSC curve for the averaged pinhead structure after classification (resolution: 23.1 Å).
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Figure 2—figure supplement 14. The anisotropic resolution in the pinhead structure. The distal tip of PinB is flexible exhibiting the lowest resolution in the average (arrowhead). The local resolution is estimated by blocres in Bsoft.
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Figure 3. The Structure of A-C Linker. (A) A top view of the A-C linker structure with its linked A- and C-tubules. The A-C linker is highlighted in red. Three filamental structures f1, f2 and f3 making up the central trunk running in longitudinal direction are marked with *. (B) The A-C linker viewed from outside of procentriole. The dashed square highlights the area that will be viewed in (D). (C) The A-C linker viewed from the luminal side of procentriole. Three filaments f1, f2 and f3 that make up the central trunk are marked with vertical dashed lines. They are interconnected and form a spiral. (D and E) Close up views of the Leg B in the A-C linker show a possible location of POC1. (D) is in side view from outside of the procentriole. (E) is in top view from the distal end of the procentriole. An atomic model of WD40-ß-propeller domain (PDB ID 1S4U) is fitted into the doughnut-shaped density in Leg B. The red * indicate connecting points of this WD40 domain to the rest of Leg B structure and to the f3 in central trunk.

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Figure 3—figure supplement 1. Schematic diagram shows the classification process for identifying intact A-C linker associated with the A-tubule. The number of subtomogram in each class and the four classes identified with intact A-C linker are indicated. Total 3992 subtomograms from four classes.
are identified with relative intact A-C linker. Among them, 2917 (73.1%) are from the proximal end of mother centrioles. 1075 (26.9%) are from the procentrioles.

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Figure 3—figure supplement 2. Subtomogram average of the A-tubule with associated A-C linker. This is based on 3992 subtomograms (Class 1–4) identified in classification scheme in Figure 3—figure supplement 1.

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**Figure 3—figure supplement 3.** FSC curves for the averaged A-tubule and the C-tubule with more intact A-C linker after classification. The A-tubule with associated A-C linker (red, 23.1 Å), the C-tubule with associated A-C linker (green, 23.1 Å).

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Figure 3—figure supplement 4. Schematic diagram showing classification process for identifying intact A-C linker associated with the C-tubule. The number of subtomogram in each class and the six classes identified with relative intact A-C linker are indicated. The Class one is not used for further refinement and average due to its low quality. Total 3478 subtomograms from six classes are identified with relative intact A-C linker. Among them, 2611 (75.1%) are from the proximal end of mother centrioles, 867 (24.9%) are from the procentrioles.

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Figure 3—figure supplement 5. Subtomogram average of the C-tubule with associated A-C linker. This is based on 3245 subtomograms (Class 2–6) identified in classification scheme in Figure 3—figure supplement 4.

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Figure 3—figure supplement 6. The scheme for Reconstruction of Full A-C Linker Structure. By using this scheme, based on the 4 and 6 classes identified (illustrated in Figure 3—figure supplements 1,4), total 10 structures with full A-C linker are reconstructed showing different degrees of the

Complete A-C linker
Figure 3—figure supplement 6 continued

twist motion. The Class 2 (568 subtomograms) identified in the second classification scheme (Figure 3—figure supplement 4) is used as an example shown in Figure 3.

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Figure 4. The Twist of A-C Linker Drives Procentriole Triplets in Iris Diaphragm Motion. (A) Schematic diagram of two triplets connected by the A-C linker. $\theta$ is defined as the angle between the Arm A and the Leg B. The results of mapping and angle measurement as shown in (B-C, Table 2) are consistent with a twisting motion of two triplets as indicated by two curved arrows when moving longitudinally from proximal to distal direction. The pivot point of this twist is at the A-C linker as indicated by an arrow. (B and C) Weighted averages of twist angle along the triplet wall in two classification schemes shown in Figure 3—figure supplements 1, 4. Six points along 90 nm longitudinal length of centriole are sampled starting from the proximal end. The weighted average of twist angle $T_i$ at point $i$ is defined as: $T_i = \frac{\sum \theta_j N_i}{\sum N_i \cdot N_j}$, $N_j$: number of subtomogram belong to class $j$ at point $i$, $\theta_j$: twist angle as defined in (A) for class $j$. (D and E) Overlay of two sets A-C linker structure based on two classification schemes (Figure 3—Figure 4 continued on next page).
Based on its longitudinal position, each structure is colored following a ‘heat map’ scheme. Arrows point to the pivot point, the central trunk.

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Figure 5. Variation of the Tubule Length in Procentriole Triplets and Detecting the B-tubule Intermediate. (A) Two examples of procentriole in tomogram. In each, two triplets marked by yellow rectangle box are selected to depict tubule length variation as shown on the right in the longitudinal
view. The distal end of each tubule is indicated by an arrowhead. Black arrows indicate examples of the slightly flared morphology at the growing end of the tubule. Scale bar: 100 nm. (B) Histogram showing length distribution for A-, B- and C-tubules. For each tubule, the average length, its standard deviation and the number of measurement are indicated. (C) The averages of incomplete B-tubule from the proximal, distal and nascent doublet, respectively. Each image is a z-projection of doublet of 9.6 nm long. The numbers of subtomogram in each class and their percentages are indicated. The arrowhead indicates PF B1. (D) Examples of incomplete B-tubule in procentriole tomogram from three classes. The incomplete B-tubules are marked by yellow arrowheads.

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Figure S5—figure supplement 1. Classification and Identification of Partially Assembled B-tubule. Total 157 subtomograms with incomplete doublet are identified as the result of classification. Images displayed are the central z-sections from the average of each class. The number of subtomogram in each class is indicated.

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Figure 6. Detecting the C-tubule Intermediate. (A) The averages of subset of subtomogram with incomplete triplet at proximal, distal end and from nascent triplet. The images are z-projection of the average where the triplet length is 9.6 nm. The numbers of subtomogram in each class and their percentages are indicated. The arrowhead points to PF C1. The arrow points to the A-C linker. (B) Examples of incomplete C-tubule in procentriole tomogram in three classes. The incomplete C-tubules are marked by yellow arrowheads. The A-C linkers are marked by yellow arrows.

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Figure 6—figure supplement 1. Classification and Identification of Partially Assembled C-tubule. Total 208 subtomograms with incomplete triplet are identified as the result of classification. Images displayed are the central z-sections from the average of each class. The number of subtomogram in each class is indicated.

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Figure 7. A Model for the MT Triplet Assembly in Procentriole. In the model, the triplet assembly can be divided into five sequential steps: 1) A-tubule emerges and anchors to the pinhead in the cartwheel. It continues to elongate longitudinally. 2) B-tubule branches out at PF A10 in outer A-B junction. 3) B-tubule expands laterally from outer A-B junction toward the luminal inner A-B junction where it completes the B-tubule. 4) C-tubule branches out at both inner and outer B-C junctions. Meanwhile, the A-C linker is established. 5) The bi-directional lateral expansion of C-tubule completes the triplet assembly at this longitudinal position.

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