A Structural Road Map to Unveil Basal Body Composition and Assembly

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Abstract

The Basal Body (BB) acts as the template for the axoneme, the microtubule-based structure of cilia and flagella. Although several proteins were recently implicated in both centriole and BB assembly and function, their molecular mechanisms are still poorly characterized. In this issue of The EMBO journal, Li and coworkers describe for the first time the near-native structure of the BB at 33 Å resolution obtained by Cryo-Electron Microscopy analysis of wild type (WT) isolated *Chlamydomonas* BBs. They identified several uncharacterized non-tubulin structures and variations along the length of the BB, which likely reflect the binding and function of numerous macromolecular complexes. These complexes are expected to define BB intrinsic properties, such as its characteristic structure and stability. Similar to the high-resolution structures of ribosome and nuclear pore complexes, this study will undoubtedly contribute towards the future analysis of centriole and basal body biogenesis, maintenance and function.

Main text

The microtubule (MT)-based structure of the cilium/flagellum grows from the distal part of the BB, which in many animal cells develops from the mature centriole in the centrosome. Electron microscopic (EM) images of chemically fixed resin-embedded centrioles and basal bodies (CBBs) suggest that their ultrastructure is similar, and that their key components are MTs. The mechanisms underlying the organization of CBB MTs are likely to hold many surprises as they are remarkably different from other MTs in the cell, comprising closed and open MTs and being highly stable. Additionally, non-MT-based structures are also part of the CBB, including a cartwheel in the proximal lumen region that reinforces CBB symmetry (*reviewed in* Carvalho-Santos et al., 2011; Azimzadeh et al., 2010).

Several centriole components and BB proteins were identified by comparative and/or functional genomics and proteomics studies of purified CBBs (*reviewed in* Carvalho-Santos et al., 2011; Azimzadeh et al., 2010). Advances in our understanding of the molecular mechanisms of CBB assembly depend on high-resolution comparative studies of WT and mutant structures, as well as characterization of the localization of molecular complexes within the small CBB structure. Despite the existence of beautiful ultrastructure data based on chemically-fixed specimens (Geimer & Melkonian, 2004; Ibrahim et al., 2009), high-resolution structures of native CBBs were missing. Using electron cryo-tomography and 3D sub-tomogram averaging, Li and colleagues solved the structure of the near-native BB triplet at 33 Å resolution (Li et al., 2011). A pseudo-atomic model of the tubulin protofilaments at the core of the triplets was built by fitting the atomic structure of α/β-tubulin monomers into the BB tomograms.

The 3D density map reveals several additional densities that represent non-tubulin proteins attached, both internally and externally, to all triplet MTs, some linking two MTs of the triplets and/or consecutive triplets (Li et al., 2011; for a summary see Table 1). These structures are likely composed of several proteins that have previously been isolated with CBBs. A Y-shaped structure and large rod-shaped structures emanate from the triplet A/B- and C- tubules, respectively, and extend towards the BB central lumen. Possibly, these large inner circular structures in the BB lumen function as a scaffold that stabilizes the entire BB barrel (Li et al., 2011; Figure 1; Table 1). Linker structures had been observed before (eg. Ibrahim et al., 2009;

Geimer & Melkonian, 2004), but with less detail and complexity. In this work it is speculated that some of the additional densities present at the A- and B-tubule inner wall are due to the tektin family proteins, which may confer rigidity to the BB triplet (Amos, 2008).

The authors also describe that the BB proximal and distal structures are significantly different. The majority of the changes are confined to 1) the C-tubule, 2) linkers between the adjacent triplets and 3) the twist angle of the triplets along the BB length (Li et al, 2011; Table 1; Figure 1). It is possible that together with the cartwheel, the linkers between consecutive triplets contribute to establishing and reinforcing the CBB nine-fold symmetry, by defining the angles between triplets and in consequence the available space to fit these MTs. The authors also propose that the structural variations along the length of the BB suggest a sequential and coordinated BB assembly process. It will be important to obtain high-resolution structures of the growing WT CBB and that of mutants in genes associated with CBB stability and elongation, such as δ- tubulin, POC5, CPAP, POC1 and Bld10 (*reviewed in* Azimzadeh et al, 2010; Carvalho-Santos et al., 2011) to complement previous work (Pelletier et al, 2006; Guichard et al, 2011) and unveil CBB assembly mechanisms.

A comparison of the BB structure with that of the axoneme (resolved at 30 Å; Sui & Downing, 2006) revealed that the distribution of the accessory structures on the outer and inner surface of the A- and B- tubules of the BB triplet are different from the axonemal doublet MTs they template (Li et al, 2011). It will be important in the future to understand what those differences mean for CBB and axoneme function, including the links with pericentriolar components and motility.

The high-resolution structure of ribosome and nuclear pore complexes solved by single particle reconstruction electron cryo-tomography contributed immensely to our knowledge on these organelles assembly and function (*reviewed in* Ramakrishnan, 2009; Ben-Harush et al, 2010). The BB high-resolution structural analysis reported in this article (Li et al, 2011) will certainly pave the road for the identification of essential non-MT BB components, and allow us to understand their molecular role in the context of CBB biogenesis, maintenance and function.

Figure 1:

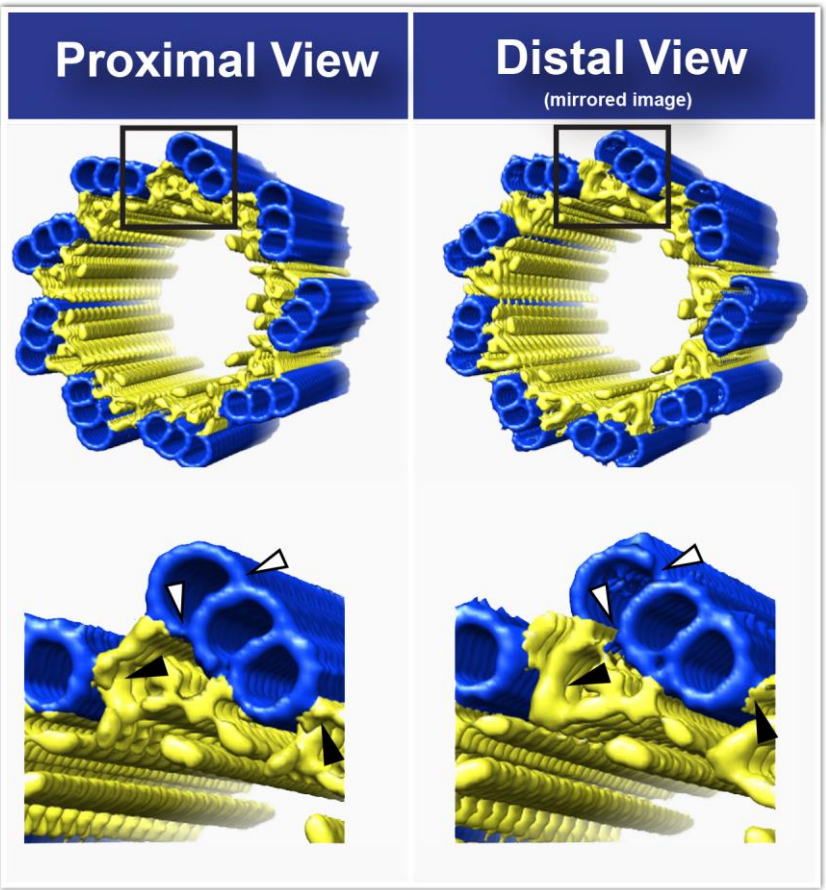


Figure 1: Proximal and distal views of the reconstructed basal body model. MT triplets are represented in blue, and non-tubulin proteins attached to the triplets are represented in yellow. Note the structural differences between the proximal and distal regions of the BB at the level of the C-tubule and non-tubulin structures. Lower images represent 3x magnified view of the box marked area; white arrowheads – indicate the changes in the C-Tubule configuration; black arrowheads – indicate changes in the non-MT structures. Distal view is mirrored to facilitate the comparison with proximal view. Images were kindly provided by Sam Li.

Table 1:

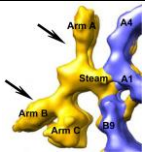
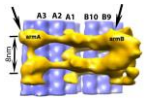
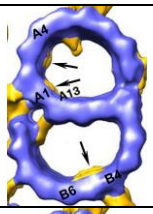
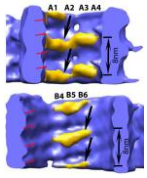
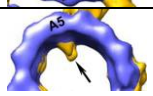
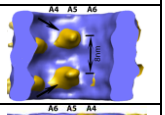
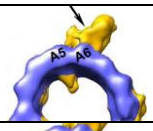
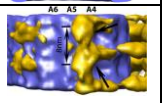
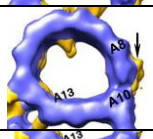
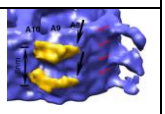
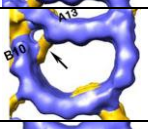
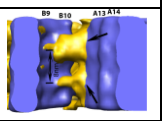
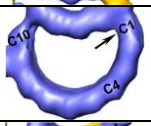
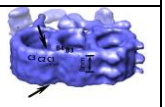
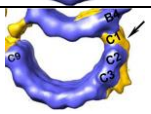
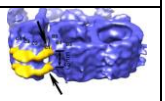
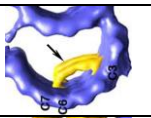
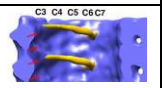
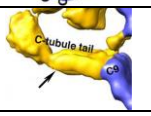
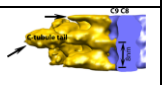
Non- α/β-tubulin structures		Present at	Periodicity along the length	Estimated mass	Predicted molecule	Suggested function	Top View	Horizontal View
Y-shaped structure	Central stem	Junction of A1/2 and B10	8nm 16nm (fused part of ArmB)	1.1 MDa	--	1. Forms a scaffold on the luminal side. 2. Cross-links and stabilizes A- and B-tubules.		
	Arm A							
	Arm B							
	ArmC							
Small structures along the internal wall of the A- and B-tubules		1.A1- A4 2.B4-B6	8nm	--	Tektins	Laterally cross-links neighboring tubulin monomers		
Cone shaped structure on the internal wall of the A-tubule		A5	8nm	--	--	Stabilizes the A-tubule		
Cone shaped structure on the luminal side of the A- tubule		A5/6	8nm	--	--	Helps to connect the A-tubule to the neighboring triplet		
Small structure on the outside wall of A-tubule		A8-A10	8nm	--	--	Stabilizes the linkage between A- and B-tubules		
Filamentous ladder-like structure inside the B-tubule		B10 and A13	4nm	--	--	Stabilizes the linkage between A- and B-tubules		
Globular structure at C1 (Proximal part of BB)		C1	4nm	50 kDa	δ-tubulin	Helps to extend and stabilize the C-tubule		
Hook shaped arm structure (Distal part of BB)		1.C1 2. C2-C4 lateral surface	8nm	270 kDa	--	Stabilizes the linkage between B- and C-tubules		
Crescent-shaped filamentous structure (Distal part of BB)		C3 to C6/7 junction	8nm	46 kDa	--	Enhances the rigidity of the C-tubule in the distal half of the BB		
Large C-tubule tail		C9	--	--	--	Stabilizes the inner barrel		

Table 1: Characteristics of the non-α/β -tubulin structures reported in Li et al, 2011 in this issue of EMBO journal. Arrows mark the regions of interest. BB, Basal Body. All the Images were adapted from Li et al, 2011 or kindly provided by Sam Li.

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