Cryo-electron tomography reveals conserved features of doublet microtubules in flagella

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AUTHOR SUMMARY

Eukaryotic cilia and flagella are thin hair-like appendages on the surface of most animal and lower plant cells. Cells use these organelles to generate movement and to sense and respond to the environment. Defects in cilia and flagella are known to cause many diseases and developmental disorders (ciliopathies) in numerous organisms, including humans, such as polycystic kidney disease, respiratory disease, and neurological disorders (1). An essential feature of these organelles is the presence of nine outer doublet microtubules (hollow protein tubes) that form the cylindrical core of the structure known as the axoneme. Biochemical and structural evidence indicates that the molecular architecture of doublet microtubules has been conserved in eukaryotes since their evolution ~850 Mya (2). The doublet microtubule is formed by tubulin protofilaments and other structural proteins, such as tektins, which provide a scaffold for the attachment of dynein motors (responsibility for driving ciliary and flagellar motility) and regulatory components in a highly specific and ordered manner. Despite the importance of doublet microtubules for the motility of cilia and flagella, long-standing questions and controversies about their assembly, stability, and detailed structure remain. A greater appreciation of the assembly and function of cilia and flagella as well as their role in disease therefore requires a better understanding of their fundamental structure. In this study, we used a high-resolution imaging technique, cryo-electron tomography (cryo-ET), to gain unique insights into the structure of doublet microtubules from Chlamydomonas (single-celled algae) and sea urchin sperm flagella.

Cryo-ET allows the visualization of the 3D structure of life-like preserved cells and organelles; the experimental steps include the following: (i) rapid freezing of the sample to cryo-immobilize the molecules without formation of damaging ice crystals; (ii) tilting the specimen in the electron microscope to collect ~70 different views from +65° to −65°; (iii) computational alignment of the views to calculate a 3D reconstruction, called the tomogram, of the imaged sample; and (iv) computational averaging of repeating structures in the tomogram to reduce noise and increase resolution. Cryo-ET provided the necessary resolution to show that the B-tubules of doublet microtubules are composed of 10 protofilaments, rather than 11, and that the inner and outer junctions between the A- and B-tubules are fundamentally different (Fig. P1). The outer junction, crucial for the initial formation of the doublet microtubule, appears to be formed by interactions among the tubulin subunits of three protofilaments with unusual tubulin interfaces, but one of these protofilaments does not fit with the conventionally accepted orientation for tubulin protofilaments. This outer junction is important physiologically, as shown by mutations affecting the usual pattern of chemical (posttranslational) modifications of tubulin (3). In contrast, the inner junction is not formed by direct interactions among tubulin protofilaments. Instead, a ladder-like structure that is clearly thinner than tubulin connects protofilaments of the A- and B-tubules (Fig. P1). The recently discovered microtubule inner proteins (MIPs) on the inside of the A- and B-tubules (Fig. P1) are more complex than previously thought. MIP1 and MIP2 are both composed of alternating small and large subunits recurring every 16 and/or 48 nm along the inner A-tubule wall. We found that MIP3 forms small protein arches connecting the two B-tubule protofilaments closest to the inner junction but does not form the inner junction itself. MIP4 is associated with the inner surface of the A-tubule along the partition protofilaments (i.e., the 5 protofilaments of the A-tubule bounded by the 2 junctions with the B-tubule). Finally, we observed another structure called “beak-MIP” that is present within a specific subset of B-tubules of Chlamydomonas doublet microtubules and is composed of a longitudinal band of proteins repeating every 16 nm.

These structural findings are highly relevant to ongoing genetic and biochemical studies focused on understanding the mechanisms of assembly and motility of cilia and flagella, their roles in signal transduction, and their roles in various diseases and developmental disorders (4). The present cryo-ET results clearly demonstrate that there are 10 protofilaments in the B-tubule, revealing a high degree of conservation in the fundamental assembly and structure of eukaryotic microtubules.

Author contributions: D.N. designed research; D.N., X.F., T.H., and A.T. performed research; M.E.P. contributed new reagents/analytic tools; D.N., X.F., T.H., and R.W.L. analyzed data; and D.N. and R.W.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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See full research article on page E845 of www.pnas.org.

Cite this Author Summary as: PNAS 10.1073/pnas.1106178108.
mental structure of flagella. This is important because the B-tubule provides the track for the dynein motors that drive ciliary and flagellar motility. The image resolution is sufficient to reveal evidence for both a nontubulin inner AB-junction and unique tubulin protofilament interactions at the trimeric outer AB-junction, both of which are physiologically important for B-tubule assembly. Finally, the detailed observations on the various MIPs provide a new avenue for the exploration of these evolutionarily conserved and critical structural components.

The high degree of conservation in flagellar structure, as demonstrated in this study, is evidence of the importance of these structures in eukaryotic form and function. Indeed, mutations affecting the posttranslational modification of tubulin disrupt the formation or closure of the outer junction (3), and mutations in the gene encoding a G protein disrupt a specific signaling pathway in mice, leading to multiple embryonic developmental defects and failure to form the inner AB-junctions (5). The structural findings described here, together with previously published biochemical and genetic data, provide a solid foundation for future work on the molecular assembly and stability of the doublet microtubule and axoneme, and they should advance our understanding of the molecular mechanisms of ciliary and flagellar motility and signal transduction in normal and disease states.