In Focus

Sculpting the dynein regulatory complex
Cryoelectron tomography materializes the (missing) nexin link.

If a picture paints a thousand words, the text generated by cryoelectron tomography (cryo-ET) could fill volumes. High resolution 3D reconstructions of flagellar axonemes generated using cryo-ET are shaping our understanding of how structure relates to function, a point exemplified by a new study which assigns a molecular identity to a formerly elusive ciliary structural feature—the nexin link (1).

Cilia and flagella have many functions within the human body, including movement of extracellular fluid and mechano-sensory perception. Dysfunction of these organelles is associated with developmental defects and ciliopathy diseases, including Bardet-Biedl syndrome and polycystic kidney disease (2).

Traditional electron tomography methods provide a rough sketch of the ciliary and flagellar framework, which consists of nine doublet microtubules (a full A-tubule and an attached semicircular B-tubule) surrounding a pair of singlet microtubules. Nexin links were described in the early 1960s as circumferential connections between outer doublets (3), but their biochemical identity and function have remained obscure.

An elaborate pattern of motor and regulatory proteins repeats every 96 nm along the length of each doublet, including the outer and inner dynein arms, a pair of radial spokes, the nexin links, and the dynein regulatory complex (DRC). Fixation techniques such as rapid freezing combined with cryo-ET allow structures that are destroyed by harsher fixation methods to be visualized with sculpture-like detail. A collaborative effort between the Nicastro lab and the Porter lab, Heuser et al. have now applied the technique to reveal the DRC’s structure and function (1).

Previous models of the DRC drew it as a crescent-shaped structure nestled between the outer dynein arms and the base of the distal radial spoke. Nicastro’s team now finds that the DRC is larger, and makes more extensive contacts with other elements of the axoneme than previously thought. Hundreds of averaged images show that the DRC is composed of a base plate and a bifurcated linker domain. The base plate spans both the A- and B-tubule of a doublet. Heuser et al. mapped 10 contact points between the DRC, the outer and inner dynein arms, and the distal radial spoke. The observation that the DRC contacts both the outer dynein arms and inner dynein arms suggests a mechanism for coordinating motor activity between them.

The new images also show that the linker extends 50 nm towards the B-tubule of the adjacent microtubule doublet. Previous studies noted the proximity of the DRC to the nexin link, but lacked the resolution necessary to unambiguously equate these structures. Sherlock Holmes was fond of saying that when all other possibilities have been eliminated, the remaining possibility must be true. As the only identifiable structure that spans the distance between neighboring doublet microtubules, the team concluded that the DRC must be the nexin link.

To date, only seven polypeptides have been identified as components of the DRC based on their absence in drn mutants. Correlation of missing densities in cryo-ET reconstructions from well-characterized mutants allows researchers to map the likely positions of the DRC subunits. For example, cryo-ET data of axonemes from Chlamydomonas pf2 mutants lacking DRC subunits 3–7 is missing the linker region, implying that DRC3–7 compose the base plate. Heuser et al. also identified structures with no known peptide correlate. They estimate the mass of the DRC to be between 1.4 and 1.5 MDa—nearly three times the sum of the seven currently known DRC subunits.

Identifying the nexin links as part of the DRC has important implications for their function. Instead of merely stabilizing the outer doublet microtubules, the expanded structure clearly illustrates that the nexin-DRC is well situated to transmit signals from the radial spokes to the inner and outer dynein arms. It therefore likely coordinates dynein activity along the length and around the circumference of the axoneme. Characterization of the remaining DRC components will be critical for a complete understanding of how DRC function impacts flagellar motility.

1. Heuser, T., et al. 2009. J. Cell Biol. doi:10.1083/jcb.200908067.
2. Fliegauf, M., et al. 2007. Nat. Rev. Mol. Cell Biol. 8:880–893.
3. Gibbons, I.R. 1963. Proc. Natl. Acad. Sci. USA. 50:1002–1010.

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