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Structural Analysis of the I1 Inner Dynein Arm Complex from *Chlamydomonas* Flagella

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Cilia and flagella are highly conserved organelles with roles in cellular movement and sensory signal transduction. They perform essential functions in the human body and ciliary defects have been linked to several diseases, including polycystic kidney disease and primary ciliary dyskinesia [1]. The core structure of cilia and flagella is known as the axoneme. Most motile axonemes consist of nine microtubule doublets surrounding a central pair of singlet microtubules. The dynein arms on each doublet are organized in a 96 nm repeat pattern along the length of the axoneme [2, 3]. We recently showed that the doublets are interconnected at the distal end of each repeat via the nexin-dynein regulatory complex (N-DRC), a key regulator of axonemal bending and motility [4]. Another large complex is located on the proximal end of the 96 nm repeat, the I1 inner dynein arm complex. The I1 dynein is the only two-headed dynein in the inner row and has both regulatory and motor functions [5]. A number of I1 components have been identified: two dynein heavy chains (1αDHC, 1βDHC), three intermediate chains (IC140, IC138, IC97), and several light chains (LC8, LC7a, LC7b, Tctex1, Tctex2b), as well as the novel accessory protein FAP120 [6]. 2D averaging of electron micrographs of chemically fixed and plastic sectioned mutant axonemes revealed the positions of the two motor domains and thereby suggested that the IC/LC complex is located at the base of the I1 dynein, in between radial spoke 1 and the outer dynein arms [7, 8]. This location is consistent with other studies showing the microtubule sliding velocities are controlled by the radial spokes and closely correlated with the phosphorylation state of IC138 [5]. More recent studies of an IC138 mutant (*bop5-2*) have now revealed that IC138 is also required for assembly of an I1 sub-complex consisting of IC138, IC97, LC7b, and FAP120, and that this sub-complex is indeed located at the base of the I1 dynein [9]. To obtain more detailed information about the specific positions and interactions of individual subunits, we have re-analyzed wild type and I1 mutant axonemes from *Chlamydomonas* using cryo-electron tomography and tomographic averaging [3]. Our 3D reconstructions provide a high-resolution view of the various sub-domains within the structure of the I1 dynein. In addition, we can now resolve delicate connections to other structures within the 96nm repeat, such as the outer and other inner dynein arms, the radial spokes, the A-tubule and even the nexin-dynein regulatory complex. Through correlation of published biochemical data and this unprecedented 3D structure, we can now localize specific I1 components, including the IC138 sub-complex. Further localization of I1 components known to be involved in dynein regulation, and their interactions outside of the I1 complex, will provide new insights into the mechanism of I1-dependent regulation of axoneme motility [10].

References

[1] M. Fliegauf et al., *Nat. Rev. Mol. Cell Biol.* 8 (2007) 880.
[2] Porter and Sale, *J. Cell Biol.* 151 (2000) F37.
[3] D. Nicastro et al., *Science*. 313 (2006) 944.
[4] T. Heuser et al., *J. Cell Biol.* 187 (2009) 921.

[5] M. Wirschell et al., *Cell. Motil. Cytoskel.* 64 (2007) 569.
[6] Ikeda et al., *Cell. Motil. Cytoskel.* 66 (2009) 448.
[7] Myster et al., *J. Cell Biology.* 146 (1999) 801.
[8] Perrone et al., 11 (2000) 2297.
[9] R. Bower et al., *Mol. Biol. Cell*. 20 (2009) 3055.
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