Tubulin family: **Kinship of key proteins across phylogenetic domains**

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Atomic structures obtained by electron microscopy for tubulin, and by X-ray crystallography for bacterial FtsZ, show that the two proteins are highly homologous. The complementarity between such high-resolution studies and low-resolution reconstructions of microtubule complexes is clear, but controversy still abounds.

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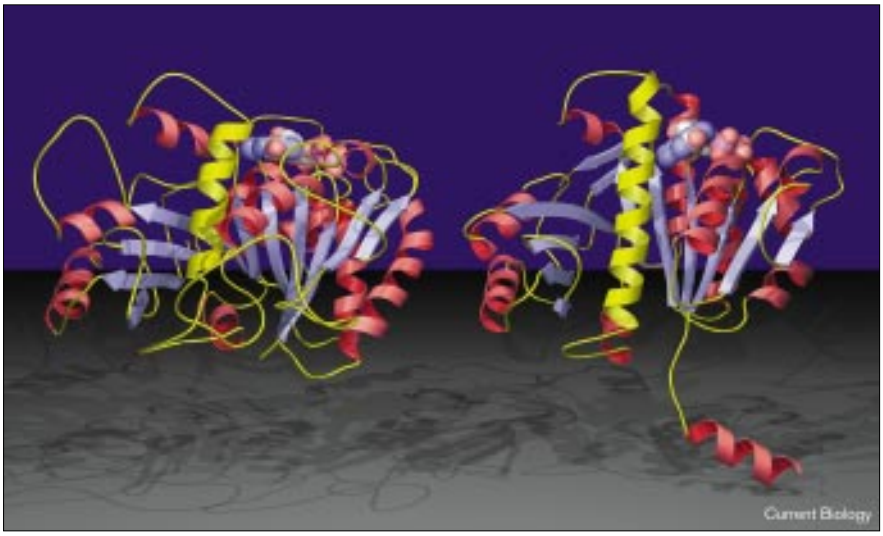
The simultaneous elucidation of the atomic structures of eukaryotic tubulin [1] and bacterial FtsZ [2] is momentous for several reasons. While limited sequence homology and a common role in cell division suggested that FtsZ might be a bacterial homolog of tubulin [3], it was not until the two structures were solved that the speculation was shown to be correct. This provides just one more example of the fact that structures are conserved far more strongly than sequences. And as the number of sequences proliferates greatly as a result of the various genome projects, it is clear that structure-based methods ([4] for example) for determining homology and function will become increasingly important. The FtsZ structure was solved at 2.8 Å by conventional X-ray crystallography [2]; somewhat surprisingly, the structure of tubulin was solved at 3.7 Å by electron microscopy [1].

Numerous attempts to obtain ordered three-dimensional crystals of tubulin had failed, in large part because the high salt, high protein concentrations needed for crystal formation cause tubulin to polymerize into helical microtubules. Such polymerization is incompatible with crystal packing and will disrupt attempts to form highly-ordered three-dimensional crystals. To surmount this problem, Downing and colleagues [1] used highly-ordered, two-dimensional zinc-induced sheets of tubulin that contain protofilaments of α and β tubulin heterodimers. These sheets were ideal candidates for high-resolution electron microscopy and served to generate the first structure of a non-membrane protein to be solved by electron microscopy. While the determination of an atomic structure by electron microscopy might come as a surprise to many in the field of cytoskeletal proteins, workers in the field of integral membrane proteins have been much more familiar with the advances in electron microscopy. Almost half of the current structures of integral membrane proteins have been obtained by electron microscopy.

The stronger conservation of structure than sequence is shown very clearly in the case of tubulin (Figure 1). While the α and β tubulin subunits show about 40% sequence identity, the two are nearly identical in structure [1]. A structural core is highly conserved in the tubulin subunits and FtsZ, but FtsZ has an amino-terminal extension absent in the tubulin subunits, and each tubulin subunit has two long, carboxy-terminal helices absent in FtsZ. These observations suggest that, within the eukaryotic

Figure 1



Ribbon models of eukaryotic β tubulin [1] (left) and prokaryotic FtsZ [2] (right). The core structures are highly homologous, with a major difference being the amino-terminal helix (bottom right) that occurs in FtsZ but not tubulin. As the amino-terminal extension does not appear to be highly conserved among the prokaryotic FtsZ proteins, Löwe and Amos [2] suggest that it is unlikely to be involved in filament formation. (Graphic courtesy of Jan Löwe.)

lineage, a more complex, heterodimeric protein evolved from a single ancestral FtsZ-like precursor.

The knowledge that FtsZ has a similar structure to tubulin does not, however, tell us what the functional form of FtsZ is within the cell [5]. For example, we know that actin, hexokinase and the ATP-binding domain of heat shock protein 70 all have a similar structure [6], despite vanishingly small sequence conservation, but only actin polymerizes to form a helical polymer. Although FtsZ has been observed to form protofilaments, tubes and sheets *in vitro*, whether the protein forms microtubule-like structures *in vivo* is still an open question. The demonstration that the *in vitro* polymerization and depolymerization of FtsZ is regulated by the hydrolysis of GTP [7], as is that of tubulin, is an important step towards establishing the likelihood that FtsZ might polymerize in a similar manner to tubulin within the cell.

The field of muscle research has been greatly advanced by the determination of atomic structures for actin [8] and myosin [9]. But it has also become apparent that these structures alone do not solve many of the vexing questions that have dominated this field. Rather, the structures elevate the plane of the debate to a much higher level. The structures provide a framework within which all biochemical, spectroscopic and genetic results can be understood. They also allow for the interpretation of low-resolution electron microscopy in terms of an atomic model. For example, different conformational states seen in F-actin by electron microscopy can now be understood in terms of the atomic structure of actin [10]. This complementarity between X-ray crystallography and electron microscopy has blossomed recently in several areas, such as virus research [11]. Similarly, the determination of structures for tubulin [1] and two tubulin-binding motor proteins, kinesin [12] and ncd [13], will not end the search for understanding the basis of tubulin-based motility. If recent papers are any indication, this area will remain quite controversial.

An attempt to fit the crystal structure of the kinesin-like protein ncd into a low-resolution three-dimensional reconstruction of a microtubule–ncd complex was reported last year by Sosa *et al.* [14]. Kozielski *et al.* [15] have now performed a similar analysis, but using a kinesin dimer crystal structure [16] and electron micrographs of microtubules decorated with such dimers. They have found a nearly opposite orientation for the motor protein to that predicted by Sosa *et al.* [14], and predict a binding stoichiometry of one kinesin dimer per tubulin heterodimer, with one head bound and the second unbound and disordered. The same binding stoichiometry was also obtained by Hirose *et al.* [17]. Kozielski *et al.* [15] conclude that kinesin dimers bind to the microtubule in a very similar conformation to that observed in the kinesin dimer crystal [16]. This would allow for a simple complementarity between the electron microscopic and X-ray crystallographic studies, as the high-resolution structures can be placed into the low-resolution reconstruction.

In contrast to Kozielski *et al.* [15], Hoenger *et al.* [18] explain their reconstruction of microtubules decorated with dimeric kinesin by a binding stoichiometry of one kinesin head per tubulin heterodimer. In this interpretation, there are no unbound heads. This view leads to a very different understanding of the relation between the crystal structure of the kinesin dimer [16] and the kinesin dimer–microtubule complex. According to Hoenger *et al.* [18], the dimer must completely open so that each kinesin head binds a tubulin protomer equivalently, and the kinesin dimer must therefore have a very different conformation in the crystal from that which it adopts when it binds to the microtubule.

Those who have followed the field of microtubule structural studies, with many reversals and conflicts concerning which is the 'plus end' and which the 'minus end' of the polymer, which subunit is α and which is β, will not be very surprised that the docking of a kinesin dimer onto a microtubule is controversial. While the field promises to generate more controversy in the future, it is clear that now the debate has been greatly transformed from one of 'blobology' to one of interpreting low-resolution three-dimensional complexes in atomic detail.

References

1. Nogales E, Wolf SG, Downing KH: Structure of the αβ tubulin dimer by electron crystallography. *Nature* 1998, 391:199-203.
2. Löwe J, Amos LA: Crystal structure of the bacterial cell-division protein FtsZ. *Nature* 1998, 391:203-206.
3. Lutkenhaus J: FtsZ ring in bacterial cytokinesis. *Mol Microbiol* 1993, 9:403-409.
4. Fischer D, Eisenberg D: Fold recognition using sequence-derived predictions. *Protein Sci* 1996, 5:947-955.
5. Lutkenhaus J, Addinall SG: Bacterial cell division and the Z ring. *Annu Rev Biochem* 1997, 66:93-116.
6. Flaherty KM, McKay DB, Kabsch W, Holmes KC: Similarity of the three-dimensional structures of actin and the ATPase fragment of a 70-kDa heat shock cognate protein. *Proc Natl Acad Sci USA* 1991, 88:5041-5045.
7. Mukherjee A, Lutkenhaus J: Dynamic assembly of FtsZ regulated by GTP hydrolysis. *EMBO J* 1998, 17:462-469.
8. Kabsch W, Mannherz HG, Suck D, Pai EF, Holmes HC: Atomic structure of the actin:DNase I complex. *Nature* 1990, 347:37-44.
9. Rayment I, Rypniewski WR, Schmidt-Base K, Smith R, Tomchick DR, Benning MM, Winkelmann DA, Wesenberg G, Holden HM: Three-dimensional structure of myosin subfragment-1: a molecular motor. *Science* 1993, 261:50-57.
10. Orlova A, Chen X, Rubenstein PA, Egelman EH: Modulation of yeast F-actin structure by a mutation in the nucleotide-binding cleft. *J Mol Biol* 1997, 271:235-243.
11. Baker TS, Johnson JE: Low resolution meets high: towards a resolution continuum from cells to atoms. *Cur Opin Struct Biol* 1996, 6:585-594.
12. Kull FJ, Sablin EP, Lau R, Fletterick RJ, Vale RD: Crystal structure of the kinesin motor domain reveals a structural similarity to myosin. *Nature* 1996, 380:550-555.
13. Sablin EP, Kull FJ, Cooke R, Vale RD, Fletterick RJ: Crystal structure of the motor domain of the kinesin-related motor ncd. *Nature* 1996, 380:555-559.

14. Sosa H, Dias DP, Hoenger A, Whittaker M, Wilson K, Sablin E, Fletterick RJ, Vale RD, Milligan RA: A model for the microtubule-Ncd motor protein complex obtained by cryo-electron microscopy and image analysis. *Cell* 1997, 90:217-224.
15. Kozielski F, Arnal I, Wade RH: Understanding how kinesin interacts with microtubules. *Curr Biol* 1998, 8:191-198.
16. Kozielski F, Sack S, Marx A, Thormählen M, Schonbrunn E, Biou V, Thompson A, Mandelkow EM, Mandelkow E: The crystal structure of dimeric kinesin and implications for microtubule-dependent motility. *Cell* 1997, 91:985-994.
17. Hirose K, Lockhart A, Cross RA, Amos LA: Three-dimensional cryoelectron microscopy of dimeric kinesin and ncd motor domains on microtubules. *Proc Natl Acad Sci USA* 1996, 93:9539-9544.
18. Hoenger A, Sack S, Thormählen M, Marx A, Müller J, Gross H, Mandelkow E: Image reconstructions of microtubules decorated with monomeric and dimeric kinesins: comparison with X-ray structure and implications for motility. *J Cell Biol* 1998, 141.