Motile organelles: **The importance of specific tubulin isoforms**

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The requirements for building flagellar axonemes and centrioles are beginning to be uncovered. The carboxyl terminus of a specific β tubulin isoform plays an important role in forming the '9 + 2' structure of the axoneme; δ tubulin plays an essential role in forming the triplet microtubules of centrioles and basal bodies.

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Current Biology 2001, 11:R419–R422

0960-9822/01/$ – see front matter

Flagella and cilia are structurally conserved motile organelles found on diverse cell types, ranging from single-celled protozoa and algae to multicellular tissues in vertebrates. They have diverse roles, for example, they can propel cells such as sperm through their environment or move fluid across a cell surface, as in ciliated epithelia such as the respiratory tract and oviduct. Monocilia on cells in the embryonic node and on kidney cells are also critically important: motile cilia in the embryonic node play a role in left–right axis determination [1–3] and in kidney function [4]. Nonmotile cilia are found in rod and cone cells of the vertebrate eye, where they may be important for transport [5].

Microtubules found in the cytoplasm, axons or spindles of eukaryotic cells occur as singlets that usually consist of 13 protofilaments. Protofilaments are made up of repeating units of α and β tubulin, organized head-to-tail to form a linear substructure. The protofilaments associate laterally to form a hollow tube, the singlet microtubule (Figure 1). One of the most striking structural features of motile cilia and flagella is the presence of nine doublet microtubules that surround two central singlet microtubules to make a characteristic '9 + 2' structure. Each doublet microtubule consists of one complete microtubule, usually containing 13 protofilaments, with an incomplete 11-protofilament structure fused to it (Figure 1).

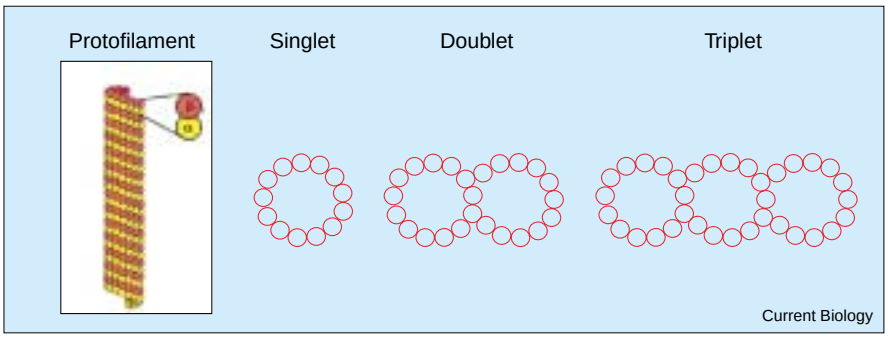
Flagella and cilia are templated by basal bodies and the doublet microtubules are continuous with the microtubules of the basal bodies. Basal bodies and centrioles are structurally related and contain triplet microtubules at their proximal end (Figure 1). The additional microtubule in a triplet also has 11 protofilaments. The mechanism by which singlet, doublet or triplet microtubules are assembled remains elusive. But recent studies, one recently published in *Current Biology* [6], are beginning to reveal the importance of specific tubulin isoforms in the assembly of these morphologically different microtubules.

Although many protozoa and algae have only a single gene each for α and β tubulin, these unicellular organisms can build singlet, doublet or triplet microtubules from a single species of α–β tubulin dimer. Clearly, other proteins or modifications must be involved in building these different types of microtubule. Multicellular organisms, in contrast, have multiple genes encoding different isoforms of α and β tubulin [7], and it has been suggested that these different isoforms may have different functions, including building distinct microtubule structures [8,9].

The specificity of different tubulin isoforms has been addressed using transgenic flies. The fruitfly *Drosophila melanogaster* has four β tubulin isoforms. Basal bodies and flagella use the same α tubulin, but different β tubulin isoforms. The β1 isoform is used for assembly of the centriole and basal body during spermatogenesis, and in nonmotile sensory cilia of other tissues [10]. The β2 isoform is used

Figure 1

The diagram on the left shows how protofilaments are arranged to form a singlet microtubule, and the arrangement of the α–β dimer. On the right are shown cross-sectional diagrams of singlet, doublet and triplet microtubules; in these diagrams, each circle represents a protofilament.



in the sperm tail, and when the gene encoding β2 tubulin is deleted, spermatogenesis fails [11,12]. Raff and colleagues [12] have suggested that the β tubulin used in motile flagella has a unique sequence motif (Figure 2): they noted that the motif D/NEEGEFDE is found in β tubulins used to assemble motile flagella or cilia, but is not conserved in the other β tubulins from the same organism or in other organisms that lack motile cilia and flagella. The β tubulins of nematode *Caenorhabdtitis elegans*, which has nonmotile sensory cilia, do not share this motif.

Raff and colleagues [6] have now dissected the role of β2 tubulin in the assembly of the 9 + 2 axonemal structure in post-meiotic germ cells, by constructing chimeras between β1 tubulin and β2 tubulin. These two tubulins differ at only 25 of 447/446 amino acids, respectively. When β1 tubulin is expressed exclusively, the flies remain infertile; they have immotile sperm, and electron microscopy showed that their sperm have the usual nine doublet microtubules, but lack the central pair microtubules. Eight of the differences between the β1 and β2 tubulins lie near the carboxyl terminus (Figure 2). When the carboxyl terminus of the β1 isoform is replaced with that of β2, flagella with a 9 + 2 axoneme are made. Even more strikingly, the replacement of just two amino acids — glutamic acid and glycine for aspartic acid and alanine — is sufficient to allow the

Figure 2

The carboxyl terminus of beta tubulin

Protozoa and animals (with motile flagella/cilia)	
eyqqyq eatad**eegefde**deegggde	*Drosophila melanogaster beta2 (85D)*
eyqqyq datie**eegefde**eeqy	*Trypanosoma brucei*
eyqqyq datve**eegefde**eeeay	*Leishmania tropica*
eyqqyq dataee**egef**eegeq	*Paramecium tetraurelia*
eyqqyq dasae**eegef**egeeeea	*Chlamydomonas reinhardtii*
eyqqyq dataee**egefde**eeegdeeaa	*Strongylocentrotus purpuratus*
eyqqyq dataee**egefde**negaegeeqpady	*Naegleria gruberi*
eyqqyq dataee**egefde**eegemgaeega	*Toxoplasma gondii*
eyqqyq datad**eegefde**demeg	*Thalassiosira weissflogii*
eyqqyq dataee**egefde**deelddamg	*Ectocarpus variabilis (brown alga)*
eyqqyq dataee**egef**eeaeeeva	*Homo sapiens beta2*
eyqqyq datadeqgefeeeegedea	*Rattus norvegicus*
eyqqyq datadeqgefeeegeedea	*Gallus gallus b2*
eyqqyq datadeqgefeeeegedea	*Homo sapiens*
eyqqyq dataeeeedfgeeaeeea	*Homo sapiens beta1*
eyqqyq dataegegv	*Homo sapiens betab4Q*
eyqqyq dataeeegemyeddeeeseaqgpk	*Homo sapiens beta4 (16)*
eyqqyq datadeqgefeeeegedea	*Homo sapiens beta (6p)*
eyqqyq dataeeeedfgeeaeeea	*Homo sapiens beta5*
eyqqyq eatadedaefeeeqeaevden	*Drosophila melanogaster beta1 (56D)*
eyqqyq eataddefdpevnqeevegdci	*Drosophila melanogaster beta3 (60C)*
Plants (without flagella or cilia)	
eyqqyq datvgeeeyeedeeeeea	*Arabidopsis thaliana 9*
eyqqyq datadeegeydveeeeegdyet	*Arabidopsis thaliana 5*
eyqqyq dataeeyeeeehdgeeeha	*Zea maize*
Fungi (without flagella or cilia)	
eyqqyq dasisegeeeyleeeeplehee	*Aspergillus viridinutans (benA)*
eyqqyq easvddeameddaeaeggagqneaveef	*Physarum polycephalum*
eyqqyq datadeegeyedeeegdlqd	
eyqqyq dagvdeeeeeyeeeaplegee	*Neurospora crassa*
eyqqyq eatveddeevdengdfgapqnqdepitenfe	*Saccharomyces cerevisiae*
eyqqyq eagidegdedyeieeekepley	*Schizosaccharomyces pombe*
eyqqyq natvddedmeyedelpledem	*Geotrichum candidum*
eyqqyq easideeeleyadeipledaame	*Candida albicans*
Animal (without motile cilia or flagella)	
eyqqyq eaaadedaaeafdge	*Caenorhabdtitis elegans*
eyqqyq eaaadedaaeafdge	*Caenorhabditis elegans (mec-7)*
eyqqyq eaaadedaaeafdge	*Caenorhabditis elegans*

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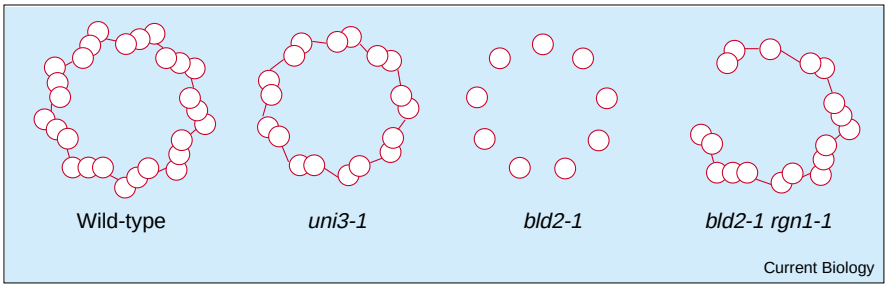
Carboxy-terminal sequences of β tubulins from a variety of species. Nielsen *et al.* [6] have shown that the carboxy-terminal motif eegefde (red) is needed to assemble the two central pair microtubules in the axoneme. Organisms that lack cilia and flagella, or lack motile cilia, lack this motif. The motif eyqqyq (blue) is common to β tubulins, and was used to align the sequences. The eegefde motif is present in most β tubulins expressed in ciliated or flagellated tissues or organisms; the eg motif that is necessary for building the 9 + 2 axoneme is highlighted in bold.

Figure 3

The microtubules of *Chlamydomonas reinhardtii* basal bodies from wild-type, *uni3, bld2* and *bld2-1 rgn1-1* cells. The *UNI3* locus encodes δ tubulin, but the gene products of the *BLD2* and *RGN1* loci are not known.



assembly of 9 + 2 axonemes. This short motif is thus required for the assembly of the central pair microtubules.

The assembly of 9 + 2 axonemes is not, however, sufficient to restore fertility to the flies. The flies producing the chimeric β tubulin fail to maintain the integrity of the distal axonemal microtubules. The results of Nielsen *et al.* [6] suggest that additional motifs in the carboxyl terminus of β2 tubulin, and in the remainder of the protein, are needed to build stable axonemes. Small sequence substitutions are clearly not sufficient to restore structural integrity to the axoneme. One could imagine that this could be either because different β tubulin isoforms form different lattices in singlet, compared to doublet, microtubules [13], or because different proteins associate with singlet or doublet microtubules to form these stable structures. Sensory cilia in *Drosophila* lack the central pair microtubules and do not require the carboxy-terminal motif of β2 tubulin for assembly.

Another conserved structural feature of cilia and flagella is the presence of basal bodies and the ability to assemble doublet and triplet microtubules. A number of additional members of the tubulin superfamily have been identified recently, and one of these, δ tubulin, has been shown to have a critical role in the formation of triplet microtubules. In the *uni3-1* mutant strain of the green alga *Chlamydomonas reinhardtii*, deletion of the δ tubulin gene results in basal bodies that have only doublet microtubules [14]. A similar phenotype has been observed in *Paramecium* after depletion of δ tubulin by gene silencing [15]. It is not clear what role δ tubulin plays in the assembly of triplet microtubules; it may act as a nucleator at the proximal end of the microtubule, or it may recruit other essential factors. A comparison of the sequence of δ tubulin to the solved structures of α and β tubulin suggests that δ tubulin may be localized to the minus, or proximal, end of microtubules [16].

In *C. reinhardtii bld2* mutants, only a ring of singlet microtubules is assembled, which is approximately one-tenth of the normal basal body length (Figure 3). These mutant cells fail to build flagella [17]. The Bld2 protein is thus needed for both doublet and triplet microtubule formation and elongation of the basal bodies. An extragenic suppressor at the *RGN1* locus restores the ability to assemble flagella to some of the cells. Examination of the basal bodies with electron microscopy shows that many of the basal bodies have less than nine microtubules, and the structures that are present have a combination of singlet, doublet and triplet microtubules (Figure 3) [18]. In rare flagella, seven or eight doublet microtubules are observed [19]. These mutant cells have thus lost the normal symmetry of the axonemal doublets. It is possible that this loss arises from an instability of the doublet microtubules.

In *Drosophila* that express β1 and β2 tubulin in a 2:1 ratio in the postmeiotic germ cells, an additional tenth doublet is observed [12]. The authors suggest that β1 tubulin in combination with β2 tubulin creates a unique templating mechanism for the formation of these new doublets. It seems possible, however, given the structural instability of axonemes at the distal end that the tenth doublet microtubule is actually generated by broken fragments that have been integrated into the axoneme.

Cilia and flagella are complex molecular machines that have over 250 different proteins [20] and move in complex patterns. These complex structures are highly conserved. Mutations that abolish the ability to assemble central pair microtubules, or substitute singlet or doublet microtubules for triplet microtubules, illustrate the complexity of elements needed to build these highly conserved and complex structures. Continued genetic analysis may provide us with a complete understanding of the role of tubulins and associated proteins in building cilia and flagella.

Acknowledgements
I thank Naomi Morrissette for critical reading and help with the figures.

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