Commentary

Protofilaments and Rings, Two Conformations of the Tubulin Family Conserved from Bacterial FtsZ to α/β and γ Tubulin

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THE tubulin family now includes α- and β-tubulin, which are the main subunits of eukaryotic microtubules; γ-tubulin, which nucleates these microtubules and regulates their dynamics at the minus end (11, 17); and FtsZ, a prokaryotic homolog of the tubulins that is the major cytoskeletal protein in bacterial cell division (5). Two new members, δ- and ε-tubulin, have been inferred from sequences in databases (2). Both α/β-tubulin and FtsZ (6) assemble into straight protofilaments that can associate further to make two-dimensional (2-D)[1] protofilament sheets. Both types of protofilaments can also adopt a curved conformation, forming small rings or spirals. The family of tubulin rings was extended recently by the discovery that γ tubulin also forms small spirals (16, 17, 25). We propose here that both protofilaments and rings are formed by all members of the tubulin family, and that they are structurally homologous across the family. This inspires a new model for how γ-tubulin rings might nucleate microtubule assembly.

The Lattice of the Microtubule Wall

The structure of the microtubule is diagrammed in Fig. 1. The wall is a 2-D polymer of subunits connected by two types of bonds. Longitudinal bonds connect alternating α- and β-subunits into protofilaments and lateral bonds connect subunits in adjacent protofilaments. When a flattened microtubule wall is viewed with the protofilaments vertical, the lateral bonds form a line of subunits with a 10° pitch from the horizontal (Fig. 2), which forms a shallow helix in the intact microtubule. This is called a 3-start helix, because it meets the third subunit up after completing a turn, and it is necessary to start three independent helices to cover all the subunits. Lateral bonds connect primarily α to α and β to β (12, 21, 22). However this lattice cannot be continued with perfect symmetry. As shown in Fig. 1, the 3-start helix of α subunits meets a β subunit when it completes the circuit. The helix continues with a string of

βs until it meets an α subunit after the next turn. The result is a seam in the microtubule wall (Fig. 1), which has recently been visualized (12, 22).

Which subunit is at the plus end, α or β? This has been a controversial question, but the evidence is now weighing heavily toward β at the plus end. GTP coated beads, which should label the exchangeable GTP-binding site on β-tubulin, bind to the plus end (15). Kinesin, which binds primarily β-tubulin (21), forms a dense stripe at the plus end (9). Finally, beads coated with antibodies against α-tubulin label the minus end (7).

A genetic argument predating these labeling studies presents a potential contradiction. Since γ tubulin was discovered as a suppressor of a mutation in β-tubulin, this implies that γ binds directly to β. It is also known that γ-tubulin nucleates at the minus end. If the γ–β contact involves a longitudinal bond, this requires that β be the terminal subunit at the minus end, contrary to the abundant evidence in favor of β-tubulin at the plus end. The new model for nucleation resolves this apparent contradiction by proposing that the γ–β contact is a lateral bond.

Tubulin Rings Are Curved Protofilaments

Tubulin rings were discovered by Borisy and Olmsted (1) in tubulin preparations before assembly and following disassembly. It was suggested at the time that these rings might serve as a circular or helical template for nucleating the helical microtubule. In the simplest model the rings might be a pre-formed 3-start helix of subunits connected by lateral bonds. However, high resolution electron microscopy demonstrated a surprisingly different topology. The rings were seen to be continuous with the protofilaments of the microtubule wall, and were therefore subunits connected by longitudinal bonds. The earliest high resolution description was in a now forgotten paper by Warner and Satir (24), describing disassembling flagellar microtubules: "the protofilaments appear to curl and form a fountain-like array, and some broken segments form nearly complete circles." Brain tubulin rings were identified as curved protofilaments by electron microscopy during assembly (4) (Fig. 2) and disassembly (13). A recent study by cryoelectron microscopy has beautifully docu-

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Address all correspondence to Harold P. Erickson, Duke University Medical Center, Durham, NC 27710. Tel.: (919) 684-6385. Fax: (919) 684-3687. E-mail: Harold_Erickson@cellbio.duke.edu

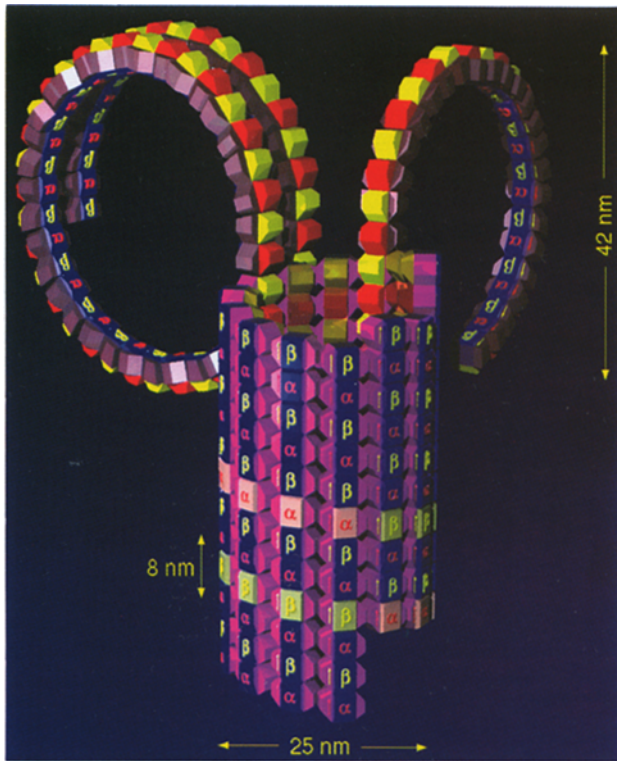1. *Abbreviation used in this paper:* 2-D, two-dimensional.

Figure 1. The microtubule lattice and the relation of rings to protofilaments. Longitudinal bonds connect alternating α and β subunits into protofilaments, and lateral bonds connect subunits into the 3-start helix, one of which is highlighted. The 3-start helix connects primarily α to α and β to β, but there is a discontinuity or seam where α connects to β. A partial ring and a spiral are shown continuous with protofilaments at the top end. The curvature of the ring is perpendicular to the plane of the microtubule wall, which means that the outside of the ring corresponds to the inside of the microtubule. This diagram and Figs. 3 and 4 were constructed with the 3-D drawing program "showcase" (Silicon Graphics).

Figure 2. The left panel shows α/β-tubulin ring polymers. Two separate closed rings are shown at top, measuring 36 and 44 nm (illustrating the range of sizes). At the bottom is a protofilament sheet showing rings continuous with the protofilaments; the arrows indicate the 3-start helix and protofilaments. The right panel shows FtsZ protofilaments and rings (average diameter 24 nm). The arrow marks continuity of a protofilament and ring. Bar, 100 nm.

mented the fountain-like array of rings at the fraying ends of microtubules (14). Note that the rings are shown curving away from the microtubule wall (Fig. 1). This orientation, the consensus from many electron micrographs (4, 13, 14), means that the outside of the ring corresponds to the inside of the microtubule.

The conclusion of all these studies is that tubulin rings are protofilaments of α/β dimers connected by longitudinal bonds. The subunits in a protofilament can exist in two conformations: a straight conformation in the microtubule wall, and a curved conformation in the ring.

FtsZ and γ-Tubulin Also Form Rings

Bacterial FtsZ can assemble in vitro into straight protofilaments and protofilament sheets, and also rings (6). The structural homology of FtsZ and α/β-tubulin rings was most strikingly demonstrated by the continuity of the curved filament with the straight protofilament in a sheet (Fig. 2). Thus FtsZ rings, like α/β tubulin rings, are curved protofilaments of subunits connected by longitudinal bonds.

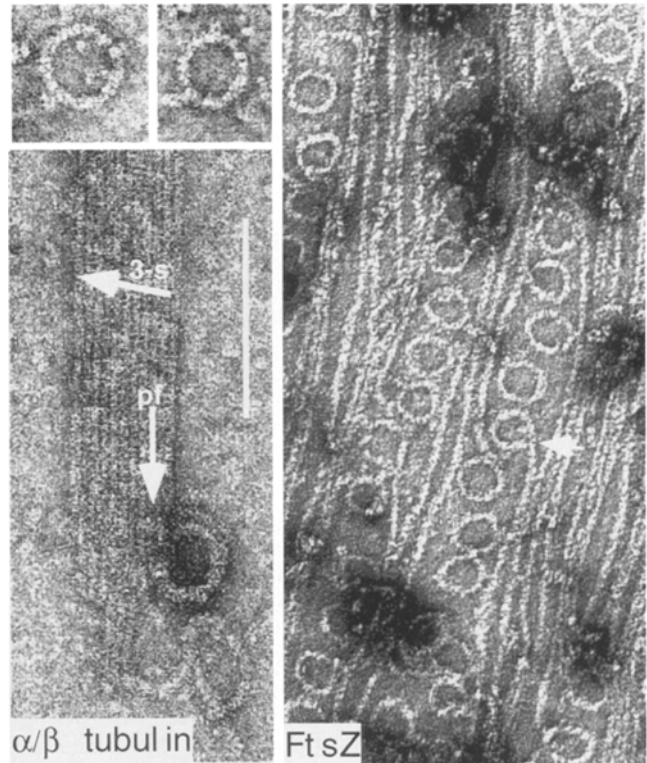FtsZ rings are only 24 nm in diameter, about half the 42

nm of α/β tubulin rings. This suggests an important insight about α/β rings. The size difference can be explained if the curved conformation occurs in all subunits in FtsZ rings (in which all the subunits are identical), but only at the β (or α, but not both) subunits in the α/β rings. This assumption is incorporated into the models in Fig. 3.

γ-Tubulin also forms rings and spirals ∼26-nm-diam (25), very similar to FtsZ rings. Micrographs have not shown the connectivity of the γ spirals, but we would have assumed it to be longitudinal, like α/β and FtsZ rings. However, accompanying the discovery of γ-tubulin rings, three papers (16, 17, 25) proposed a model in which the γ-tubulin ring corresponded to a 3-start helix, with subunits connected by lateral bonds. This would imply that the evolution of γ-tubulin has discarded the ring structure conserved from FtsZ to α/β tubulin, and re-invented a ring of the same diameter based on a radically different subunit connectivity. It seems much more reasonable that γ-tubulin rings are curved protofilaments, homologous to α/β and FtsZ rings.

Micrographs of γ-tubulin rings attached to microtubules (see Fig. 4 *b* of reference 25) actually support the longitudinal connectivity. They show the parallel to the microtubule protofilaments, similar to images of α/β rings at the end of microtubules. A 3-start helix would be perpendicular, and should present an (invisible) edge view. This lim-

Figure 3. Models of tubulin rings and spirals. Subunits in the curved conformation are modeled with a 20–23° tilt at the upper interface forming the longitudinal bond. For the α/β ring, the α subunit is assumed to be in the straight conformation and the β subunit curved. For FtsZ and γ-tubulin all subunits are in the curved conformation. The α/β ring has 16 dimers and a 41-nm outside diameter, and the FtsZ ring has 18 monomers and a 23-nm diam.

ited structural evidence, but most importantly our assumption that ring polymers are homologous across the tubulin family, support the proposal that the γ-tubulin ring is also a curved protofilament.

Microtubule Nucleation: The Protofilament Sheet, Not the Helix, Is Critical

Nucleation of microtubule assembly can occur spontaneously in a solution of purified tubulin subunits, or it can be stimulated by addition of seeds. Several studies have examined the pathway of early assembly, and conclude that the crucial event in nucleation is formation and growth of a 2-D polymer, the microtubule wall. Thus the earliest polymers are small sheets of protofilaments (3, 23). This is even the case when nucleation is seeded by stable, intact microtubules (20). The sheets grow longer by adding subunits at the end, and wider by initiating new protofilaments. The sheet has the natural curvature of the microtubule wall, and when it achieves its complement of ~13 protofilaments the edges meet and seal, presumably at a seam. By the time this closure can occur, however, the microtubule sheet has more than a thousand subunits, so the helical lattice and closure of the cylinder cannot be important in nucleation.

The nucleus has been estimated to comprise 7 (23) or 12 (8) dimers, probably arranged in two protofilaments. Once this nucleus is assembled growth will be favored, but for smaller polymers both protofilaments are unstable and disassembly is more likely than growth. We suggest that γ-tubulin might nucleate assembly by providing a stable initial protofilament that can act as a seed.

γ-Tubulin Rings: A New Model for Microtubule Nucleation

The model is diagrammed in Fig. 4. We assume that the γ-tubulin protofilament can also adopt a straight conformation (the γ-tubulin macrotubules formed in highly expressing cells probably contain sheets of γ protofilaments (19), and we assume that this γ protofilament is stable and long-lived. α/β Dimers could assemble onto this seed forming lateral bonds to γ-tubulin and longitudinal bonds to each other. Instead of having to assemble a two-protofilament nucleus as in spontaneous nucleation, with a stable γ-protofilament seed one would only need to nucleate the second protofilament. Once the second protofilament achieved its critical length the microtubule wall would spontaneously grow longer and wider, until eventually it could close to form the intact cylinder.

This model resolves the apparent conflict between the genetic evidence that suggested direct contact between γ and β, and mounting evidence that the terminal subunit at the minus end is α. Since the contacts between γ- and β-tubulin are lateral in our model, it makes no difference which subunit is at the terminus. The model of Zheng et al. (25), in contrast, postulates longitudinal contacts, which conflicts with the consensus that α is distal at the minus end.

Much recent work has focused on the role of γ-tubulin in microtubule nucleation, but earlier genetic evidence demonstrated that γ-tubulin also functions in regulating microtubule disassembly. Thus the original β-tubulin mutant *ben*A33, which was suppressed by the γ-tubulin mutants *mip*A, led to hyper-stable microtubules (18). The role of γ-tubulin in regulating the stability of the microtubule minus ends may be as important as its role in nucleation.

Conclusions and Speculations

The dual conformation of tubulin family subunits, providing a transition from a straight protofilament to a ring, appears to be conserved from bacterial FtsZ to eukaryotic tubulins. The straight protofilament is already well understood as the basis for forming the microtubule. What are the functions of rings? Nucleation of assembly seems well established for γ-tubulin rings, and α/β rings may contribute to assembly (1, 4, 13). A role in disassembly is suggested by the "fountain" of α/β rings at the ends of disassembling microtubules (14, 24). A third intriguing possibility is force generation in the transition from the straight to curved protofilament. This force may be used by depolymerizing microtubules to drag chromosomes (10), and perhaps to drive bacterial cell division (6). Tubulin rings are still obscurely understood, but the transition from straight to curved conformation is an ancient invention that may have evolved roles in several biological machines.

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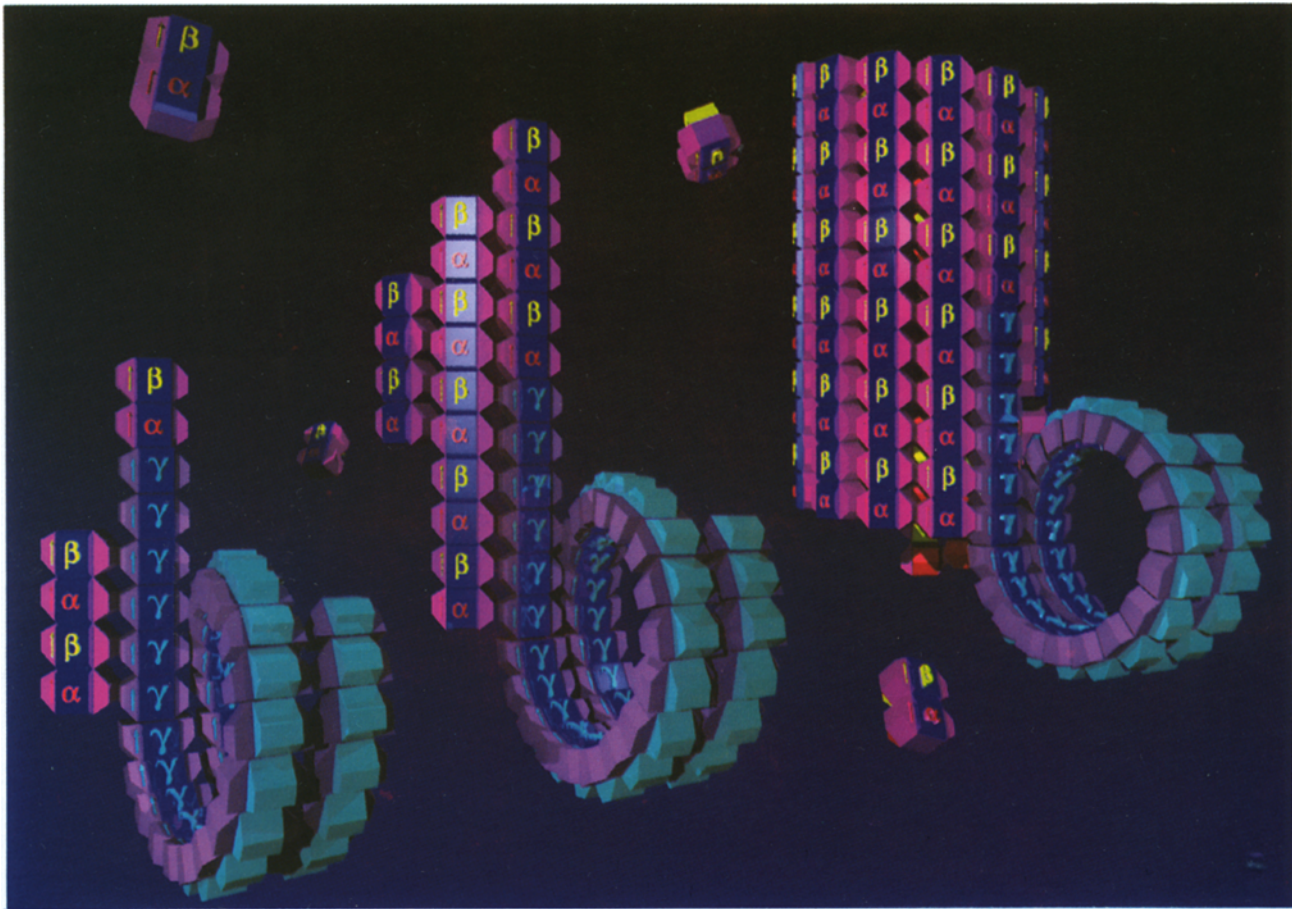4. Erickson, H.P. 1974. Assembly of microtubules from preformed, ring-

Figure 4. A model for nucleation of microtubule assembly by a γ-tubulin spiral. This spiral extends a short length of straight protofilament, which serves as a stable seed for nucleation of an α/β protofilament. α/β Subunits form lateral bonds to the γ-tubulin protofilament, and longitudinal bonds to each other. When this second protofilament has achieved three consecutive α/β subunits, growth is more favorable than disassembly (23) and the microtubule should be nucleated.

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