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New Concepts

The Nucleotide Switch of Tubulin and Microtubule Assembly: A Polymerization-Driven Structural Change†

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ABSTRACT: GTP-binding proteins from the tubulin family, including αβ-tubulin, γ-tubulin, bacterial tubulin, and FtsZ, are key components of the cytoskeleton and play central roles in chromosome segregation and cell division. The nucleotide switch of αβ-tubulin is triggered by GTP hydrolysis and regulates microtubule assembly dynamics. The structural mechanism of the switch and how it modulates assembly are beginning to be understood. A conserved structural change between the active and inactive states, different from other GTPases, may be extracted from recent tubulin and FtsZ structures. From these and the biochemical properties of tubulin, the new concept emerges that, contrary to what was thought, unassembled tubulin−GTP is in the inactive, curved conformation as in tubulin−GDP rings, and it is driven into the straight microtubule conformation by the assembly contacts; binding of the GTP γ-phosphate only lowers the free energy difference between the curved and straight forms.

Tubulin and its structural homologues play central roles in eukaryotic chromosome segregation, prokaryotic cell division, and the cytoskeleton. Eukaryotic αβ-tubulin heterodimers require GTP to assemble into microtubules made of straight protofilaments; after polymerization, the GTP bound to β-tubulin is hydrolyzed, permitting microtubule disassembly, nucleotide exchange, and the dynamics of microtubules necessary for their cellular functions (*1*, *2*). Tubulin αβ-dimers were generally believed to exist in two main states: an active GTP-bound state and an inactive GDP-state; this would be analogous to the classical nucleotide switch of GTPases. Tubulin−GTP assembles into the straight parallel protofilaments of microtubules, whereas tubulin−GDP tends to disassemble, and forms rings which correspond to curved protofilaments at depolymerizing microtubule ends (*3*−*8*). Tubulin−GDP can be driven into microtubule assembly by the binding of diverse microtubule-stabilizing agents (*9*). Prokaryotic FtsZ, which shares a limited sequence identity with tubulin, assembles into a ring structure at the cell division site (*10*), which recruits the other proteins forming the bacterial divisome and contracts during cytokinesis; chloroplasts and some mitochondria also use FtsZ to divide (*11*). Similarly to tubulin, FtsZ activates its GTPase upon cooperative polymerization forming protofilament pairs (*12*) and disassembles from the GDP form, forming curved filaments (*13*, *14*). Tubulin and FtsZ share a common structural fold in their nucleotide-binding (N-terminal) and GTPase-activating (intermediate) domains connected by a core helix (H7), and form a separate family of GTPases (*15*), distinct from the typical guanine nucleotide binding proteins which act as molecular switches (*16*). Two structures have recently been added to this protein family. The monomers of the microtubule-nucleating protein γ-tubulin bind GTP and associate in a crystal packing that replicates the lateral interactions between α- and β-tubulins in a microtubule (*17*). Bacterial

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Table 1: Structures of Proteins from the Tubulin Family and a Qualitative Assessment of Their Straight (S) or Curved (C) Conformations

protein	source	association state, ligand, nucleotide bound	resoln. (Å)	PDB ID	dimer bending	H6−H7 displacement	domain rotation
α*β*-tubulin	bovine brain	zinc-sheet polymer, taxol, GTP/GDP	3.5	1JFF[a]	S (reference)	S	S
		zinc-sheet polymer, epothilone A, GTP/GDP	2.9	1TVK[b]	S	S	S
	rat brain	RB3 domain complex, colchicine, GTP/GDP	3.6	1SAO[c]	C (reference)	C	C
		RB3 domain complex, podophyllotoxin, GTP/GDP	4.2	1SA1[c]	C	C	C
		RB3 domain complex, colchicine, vinblastine soak of 1SAO, GTP/GDP	4.1	1Z2B[d]	C	C	C
γ-tubulin	human	GTP*γ*S	2.7	1Z5V[e]	monomer	C	C
		GTP	3.0	1Z5W[e]	monomer	C	C
BtubA	*P. dejonjeii*	BtubA-thioredoxin fusion, GTP	2.5	2BTO[f]	monomer	C	C
BTubA/B	*P. dejonjeii*	BtubA/BtubB dimer, GTP/EMPTY	3.2	2BTQ[f]	C	C	C
FtsZ	*M. jannaschii*	GDP (weak *γ*-phosphate electron density)[g]	2.8	1FSZ[g]	monomer	C-like[l]	nd
		GMPCPP soak of the above	2.5	1W58[h]	monomer	C-like[l]	nd
		protofilament-like dimer, EMPTY[hh]	2.7	1W59[h]	-C[k]	C-like[l]	nd
		protofilament-like dimer, GTP soak	2.2	1W5B[h]	-C[k]	C-like[l]	nd
		protofilament-like dimer, Mg GTP soak	2.4	1W5A[h]	-C[k]	C-like[l]	nd
FtsZW319Y	*M. jannaschii*	GTP	3.0	1W5E[h]	monomer	C-like[l]	nd
FtsZ	*P. aeruginosa*	SulAΔ35-FtsZ(1−318) complex, GDP[ii]	2.1	1OFU[i]	monomer	S-like[m]	S-like[m]
FtsZ	*T. maritima*	Domain swapped dimer (loop T7 mutated, open monomers), GMPCPP	2.0	1W5F[h]	monomer	nd	nd
FtsZ	*M.tuberculosis*	lateral dimer, mol A CITRATE/mol B EMPTY	1.9	1RQ2[j]	monomer	S-like[n]	S-like[n]
		lateral dimer, soak, mol A GDP/mol B EMPTY	2.6	1RQ7[j]	monomer	S-like[n]	S-like[n]
		lateral dimer, soak, mol A GTP*γ*S/mol B EMPTY	2.1	1RLU[j]	monomer	S-like[n]	S-like[n]

[a] From ref *20*. [b] From ref *21*. [c] From ref *23*. [d] From ref *37*. [e] From ref *17*. [f] From ref *18*. [g] From ref *38*. [h] From ref *26*. [hh] Soaking into Mg GDP dissolved these crystals. [i] From ref *27*. [ii] The 3.9 Å structure of a SulA(full length)-FtsZ(1−318) complex with GTP did not refine well and was not deposited. [j] From ref *30* . [k] Curved in a direction opposite to 1SAO. [l] Outward movement of the H6−H7 loop with respect to S and by comparison to S-like. [m] H6−H7 loop retracted and by comparison with C-like. [n] Molecule A; nd, not determined.

tubulin BtubA/B from *Prosthecobacter*, closer to α*β*-tubulin than FtsZ, was presumably acquired by horizontal transfer, reversibly polymerizes into tubulin-like protofilaments, and hydrolyzes GTP (*18*, *19*). Structures from the tubulin family of assembling GTPases give insight into the mechanism of their nucleotide switch and polymer disassembly. This paper shows that a conserved structural switch may be extracted from tubulin and FtsZ structures and offers a new biochemical explanation for the nucleotide regulation of their assembly.

*Straight and Curved α*β*-Tubulin Structures: A Possible Switch*

Straight protofilaments were found in the atomic structure of zinc-induced tubulin sheets stabilized with taxol (*20*) or epothilone (*21*). These have been docked into microtubule electron microscopy maps reaching 8 Å resolution (*22*). The curved structure of two tubulin α*β*-dimers in complex with a depolymerizing RB3 stathmin-like domain and colchicine has revealed atomic details of a potential tubulin switch as it changes from straight dimers and protofilaments into curved ones (*23*), assuming that the same motions were to occur as a result of nucleotide hydrolysis in the absence of stathmin and colchicine. However, the curvature of the GTP−α-subunits (intradimer) and the GDP−*β*−subunits (between dimers) was very similar (12°). The monomer structural changes in the tubulin−RB3−colchicine complex, by comparison with the straight structure in zinc-sheets, include changes at the T5 loop, T7 loop, and H8 helix and a rotation of the intermediate domain with respect to the nucleotide binding domain (8° in α- and 11° in *β*-tubulin), coupled to a movement of the H6−H7 loop which accompanies a translation of core helix H7 along its axis of 1.5 Å in α- and 2.5 Å in *β*-tubulin (*23*). The nucleotide bound at the axial interfaces between monomers may directly control bending, and it is also possible that GTP binding locks helix H7 in the straight position. The internal monomer movements are coupled to the bending at the axial interfaces (see Figure 2 in ref *23*) and the bending to the loss of the lateral interactions between protofilaments. We think that from the available data it is not possible to decide whether any of these three structurally coupled processes comes first. It is clear that GTP hydrolysis unlocks the polymer structure, resulting in microtubule disassembly. 'Curved' and 'straight' are necessary simplifications, since intermediate bending states may exist. An analysis of the configuration of α*β*-tubulin dimers in taxol microtubules has indicated differences between both subunits and suggested a tendency toward the curved conformation after GTP hydrolysis (*24*). Recently, a 12 Å resolution map of tubulin−GDP protofilament spirals has indicated different intra- and interdimer interactions, although both are curved, and the structure of GMPCPP tubulin polymers has been interpreted in support of a sequential model in which a conformational change after GTP binding straightens the dimers enough for lateral contacts to form sheets, which later close into microtubules (*25*). The determination of atomic structures of tubulin, preferably free from assembly interfering proteins or drugs, in an active straight GTP conformation and in the curved GDP conformation, apparently poses a major challenge to the understanding of the nucleotide switch regulating microtubule assembly.

The Structures of γ-Tubulin−GTP and Bacterial Tubulin−GTP Are in a Curved Conformation

A fruitful approach toward determining the tubulin switch could involve the observation of structural differences between different nucleotide states of one of the more stable homologues. However, this has not yet been done (see Table 1).The structure of *γ*-tubulin monomers bound to GTP and GTP*γ*S has shown a curved conformation similar to the tubulin−GDP−RB3 complex, suggesting nucleotide-independent, curved-to-straight transitions in *γ*-tubulin at the microtubule minus-ends (*17*). Another intriguing case is bacterial tubulin,

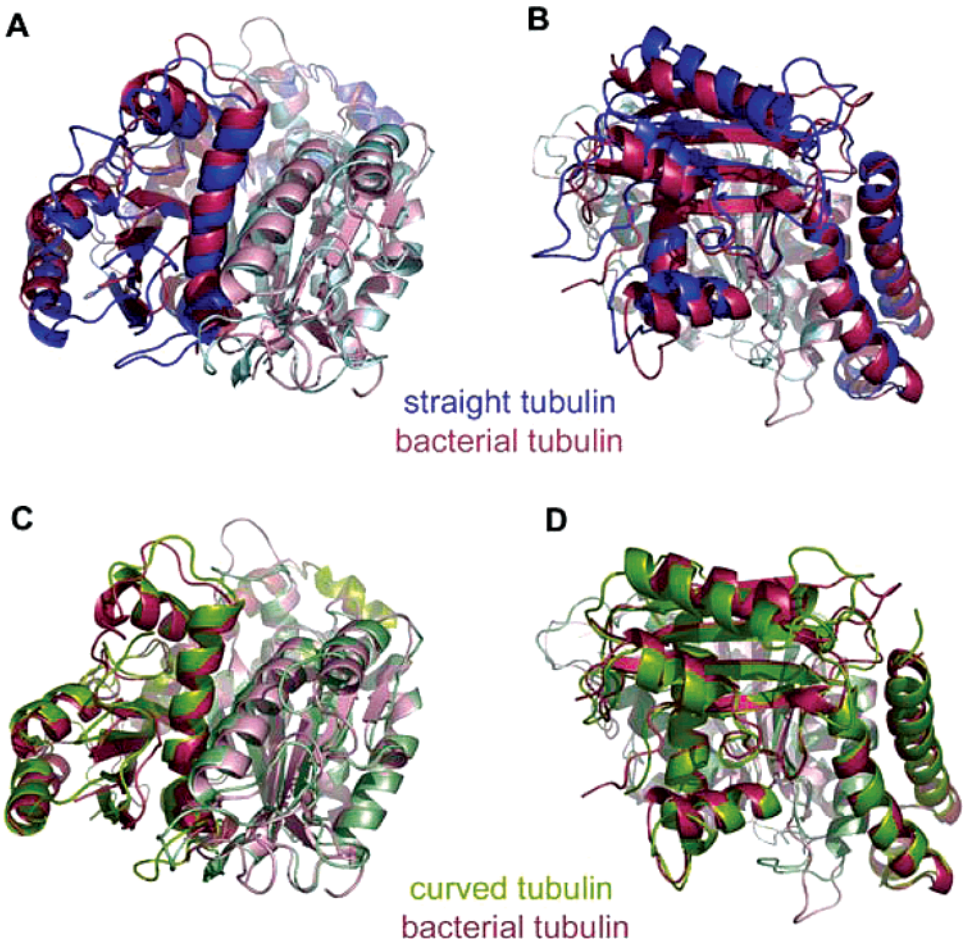

FIGURE 1: Bacterial tubulin is in a curved conformation. (A) Superposition of bacterial tubulin−GTP (red, 2BTO (A)) and straight β-tubulin monomers (blue, 1JFF (B)). The conserved N-domains were fitted and are shown in light colors (right side); the moving intermediate domain (left), central helix H7 (vertical), and short H6 (top) are in dark colors. (B) Another view of the same superposition, with the intermediate domain in front (helix H10 on top) and the C-domain helices H11−H12 on the right. (C and D) Corresponding views of bacterial tubulin (red) with curved β-tubulin (green, 1SAO (D)). Superimposed structures and alignment statistics are available as Supporting Information. The figure was prepared with PYMOL. For superposition of straight, curved, and γ-tubulin, see refs *17* and *23*.

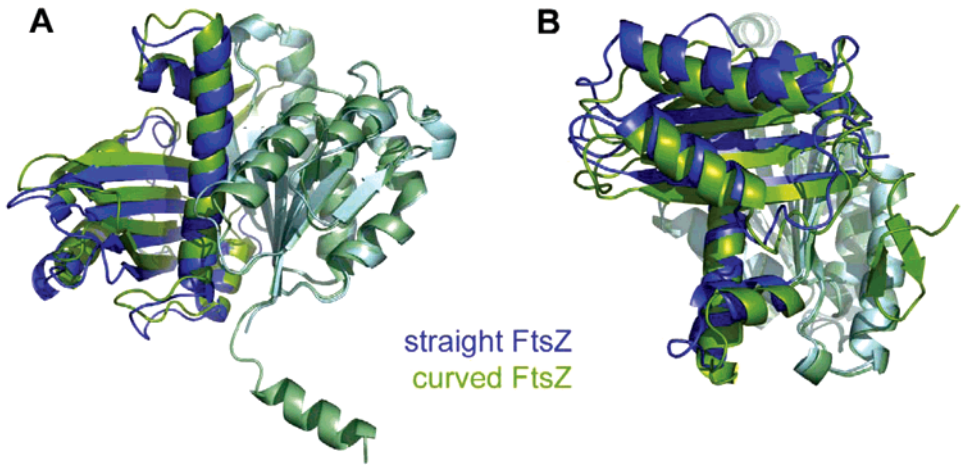

FIGURE 2: A conserved tubulin-like switch in FtsZ. Superposition of curved-like FtsZ (green, 1FSZ, including extra helix H0) with straight-like FtsZ (blue, 1OFU (A)). The superposition and views are as in Figure 1. Note the related movements in the different structures in Figures 1A,B and 2 (the intermediate domains of FtsZ are at a slightly more open angle than tubulin, and FtsZ lacks the tubulin C-domain and longer loops). See an animation in Supporting Information.

which crystallized in a curved BtubA/B heterodimer and has shown very similar GTP−BtubA, GDP−BtubA, and empty BtubB structures (*18*). Bacterial tubulin monomers show the characteristic H6−H7 displacement and an intermediate domain rotation and superimpose better onto curved tubulin, upon fitting the N-terminal domains, as depicted by Figure 1.

Curved and Straight-like FtsZ Monomers Are Independent of the Bound Nucleotide

A collection of structures of FtsZ from *Methanococcus jannaschii* (Mj), including empty, GDP-, GTP-, and GMPCPP-bound protein, showed a puzzling lack of conformational

changes (*26*), as well as a similar loop T3 conformation contrary to expectations from molecular dynamics simulations supported by fluorescence changes (*27*). Empty MjFtsZ formed curved dimers arranged in a discontinuous crystal protofilament. The structure changed very little after soaking in Mg^{2+}-GTP, revealing a complete nucleotide-binding site between FtsZ monomers; the crystals dissolved upon soaking in Mg^{2+}-GDP (*26*), in agreement with similar energetics of empty and GTP−MjFtsZ polymerization and GDP-induced depolymerization (*14*). It could be thought that these MjFtsZ structures are straight, and that a complete nucleotide-binding site is required for the bending conformational change to occur upon nucleotide hydrolysis in the filament, although the protofilament-like FtsZ dimer is bent by ≈10° (*26*) opposite to the tubulin−RB3 complex. A close inspection of the MjFtsZ monomers has revealed an H6−H7 loop position similar to the tubulin−RB3 complex (Table 1; their domain rotation was difficult to classify as straight or curved by superposition with the tubulin structures). Interestingly, upon fitting the conserved nucleotide-binding domains of MjFtsZ and *Pseudomonas aeruginosa* (Pa) FtsZ−GDP, from the complex with the polymerization inhibitor SulA (*28*), we have detected a displacement of the H7 helix and a collective movement of the intermediate domain, shown in Figure 2, which resembles that of the curved-to-straight α*β*-tubulin structures. The superimposed structures, supporting alignment statistics, and an animation of the tubulin and FtsZ switches are available as Supporting Information. The domain movement between these two structures (and between α*β*-tubulin and BtubA/B) has also been automatically detected by a program for the analysis of domain motions (*29*). The current structures in the tubulin family (Table 1) and future additions probably deserve an extensive bioinformatic analysis of their potential movements. *Mycobacterium tuberculosis* (Mt) FtsZ monomers also showed straight-like features upon superposition with MjFtsZ, although the lateral MtFtsZ dimer may be a crystallographic artifact (*30*). We have tentatively classified the PaFtsZ structure as 'straight-like' and MjFtsZ as 'curved-like' (Table 1). These two different FtsZ do not prove a structural change, but finding practically the same change in FtsZ and tubulin is striking. We suggest that these data represent the first structural evidence for a conserved nucleotide switch in prokaryotic FtsZ and eukaryotic tubulin. The movement of H7 transmits information from one monomer axial interface to the other. SulA binding, covering the T7 (bottom) interface, could straighten FtsZ by mimicking the FtzZ−FtsZ interactions in a straight protofilament. Alternately, uncomplexed PaFtsZ might prefer a straight-like conformation even when bound to GDP, or thermophilic MjFtsZ might be frozen in an inactive, curved state in solution at the crystallization temperature. Crystallization of mesophilic PaFtsZ without SulA might remove this uncertainty and perhaps reveal the FtsZ switch.

It may be considered that the available *γ*-tubulin, BtubA/B, and possibly the uncomplexed FtsZ structures, roughly correspond to the curved conformation of α*β*-tubulin, of which the tubulin−RB3 complex is a bona fide example. In contrast, the activated state of tubulin is represented by only the zinc-polymer structure, and possibly by SulA-bound FtsZ. The fact that each of the unassociated proteins crystallized in a curved conformation indicates that straight-isolated

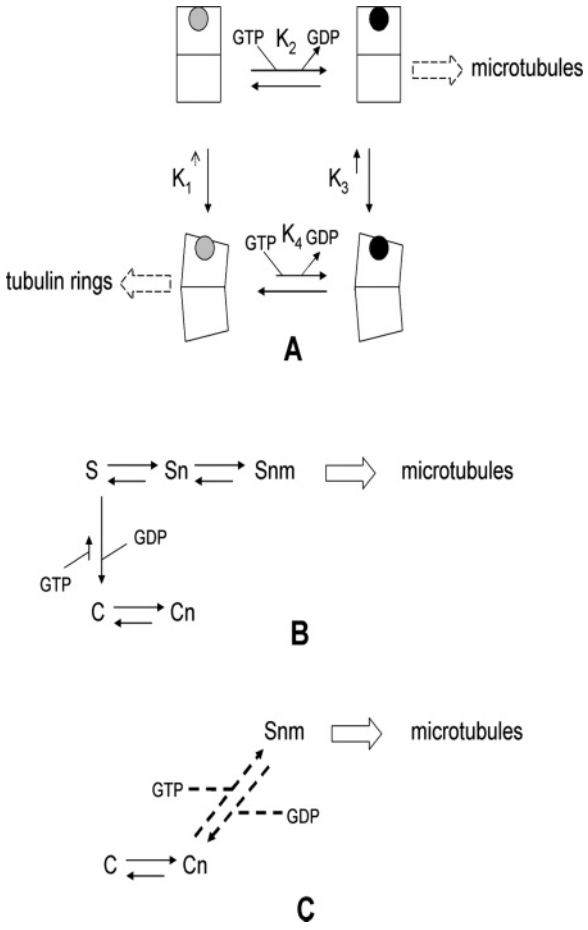

FIGURE 3: (A) Scheme of the α*β*-tubulin activation equilibria and nucleotide exchange. The GTP/GDP exchange equilibria are indicated from left to right, and the structural changes permitting microtubule assembly are indicated from bottom (ground state, curved) to top (activated, straight). The exchangeable nucleotide bound at *β*-tubulin is indicated by a dot (gray, GDP; black, GTP). It is proposed that $K_3 < 1$, $K_1 \ll 1$ (it follows that $K_2 > K_4$). Note that both tubulin−GTP and tubulin−GDP can associate into rings or microtubules, although only the preferred paths are marked. (B) A possible pathway of microtubule assembly in which activation precedes protein association. (C) A pathway in which activation is coupled to accretion and straightening of oligomers into polymerization nuclei. S, straight; C, curved tubulin dimer.

tubulin structures are infrequent, suggesting a tendency of the proteins in the tubulin family to adopt the curved conformation irrespective of the bound nucleotide. This property may appear problematic at first sight, but it is explained below.

Tubulin Conformation and Nucleotide Binding: Linked Equilibria

The proposal that the ground state of α*β*-tubulin is the curved GDP conformation (*31*) illuminates the problem of the microtubule assembly switch. The nucleotide binding and activation equilibria are schematized in Figure 3A. Equilibria 1 and 3 are the curved (ring-forming) to straight (microtubule-forming) conformational changes of tubulin−GDP and tubulin−GTP, respectively. This is a simplified scheme; the curved and straight dimer solution conformations are similar but not necessarily identical to those of associated tubulin in rings and microtubules, respectively. Equilibria 2 and 4 are the spontaneous GTP/GDP exchange (*32*) of the activated and inactive forms, respectively. Shearwin et al. (*31*)

observed that, in the absence of magnesium, tubulin−GTP or tubulin−GDP associate with the same strength into dimers, as well as into rings. They deduced that tubulin exists in the curved conformation, whether it is liganded to GDP or GTP (K_3(no Mg) ≪ 1 in Figure 3A), and proposed in their discussion that the strong binding of one magnesium ion to the straight conformation of tubulin−GTP displaces the equilibrium toward this microtubule-assembly-competent state. This would be the control mechanism for the activation of microtubule assembly, and the magnesium ion would likely be that complexed to the nucleotide γ-phosphate bound to the β-subunit (*31*). We extended the measurements of magnesium binding and linked tubulin dimerization, identified the control of the $\alpha-\beta$ dimer stability by one magnesium ion bound at the nucleotide-binding site of α-tubulin, and biochemically mapped the α- and β-tubulin nucleotide-binding sites at the intradimer and interdimer interfaces respectively (*33*). A nucleotide-dependent preequilibrium before assembly is in agreement with the observation of similar ratios between the critical concentrations of tubulin−GTP and tubulin−GDP for microtubule polymerization induced by different ligands (*9*). However, K_3 is unknown and difficult to determine; certain ligands distinguish tubulin−GTP from tubulin−GDP (*34*). Shearwin et al. (*31*) assigned a value of K_3(no Mg) = 0.01 (note that K_3(no Mg) in Figure 3A is K_1 in their Scheme II), from which they calculated a value K_3(Mg) = 16 (K_3 in their Scheme II); that is, 94% of the tubulin−GTP dimers are in the activated microtubule-forming state. Setting an equally arbitrary value of K_3(no Mg) = 0.0001 shifts the value of K_3(Mg) to 0.16 (since in their Scheme II, $K_1K_2 = K_3K_4$); that is, 86% of the tubulin−GTP dimers exist in the inactive, curved conformation. Thus, by shifting this arbitrary value in the numerical simulations (*31*) from $K_3 > 1$ to $K_3 < 1$ (Figure 3A), it is equally possible that GTP binding does not straighten dimers, but only reduces the free energy difference between the curved and straight forms; this permits straightening upon assembly. Activation with GDP is even more unfavorable, $K_1 \ll 1$, permitting microtubule disassembly upon GTP hydrolysis. Nevertheless K_1 must be different from zero, since taxol binding drives tubulin−GDP into microtubule assembly (*35*). The fact that tubulin−GDP has a stronger propensity than tubulin−GTP for double ring formation (*4*) may be explained by a better flexibility for ring closure.

Microtubule Assembly Contacts Drive Curved Tubulin into the Straight Conformation: Possible Mechanisms of GTP-Induced Microtubule Assembly

The simplest explanation for the structural comparisons and the biochemical properties taken together is that unassembled tubulin is naturally curved and adopts the activated straight conformation when it polymerizes. Unlike other GTP-binding proteins, activated unassembled tubulin−GTP is an intermediate species probably existing in small proportion in solution, hence, difficult to crystallize. Activation is induced by the binding of an 'effector', the lateral and longitudinal microtubule contacts. Tubulin−GTP differs from tubulin−GDP only in the flexibility required to adopt the straight conformation driven by the polymer contacts. Even if this was not the case and straight tubulin−GTP predominated over curved tubulin−GTP, it would not accumulate in a concentrated solution, but assemble; crystallization of straight unassembled tubulin would require blocking, if possible, polymerization but not the activation structural change. The atomic mechanism of the nucleotide switch may indeed imply the movements described for the tubulin−RB3 complex (*23*); the proof requires new structures overcoming the current limitations (different complexes or proteins and limited resolution in microtubule structures).

This new concept of activation driven by polymerization (based on structure and equilibrium) does not discriminate a kinetic mechanism for GTP-induced microtubule assembly. Two pathways may be envisaged, depending on when the tubulin molecule acquires the straight microtubule conformation. Along one pathway (Figure 3B), the conformational change precedes association of a small proportion of straight dimers (S) into straight linear oligomers (Sn), which are selected for two-dimensional polymerization (Snm), displacing the preceding unfavorable equilibrium from the curved to the straight conformation. This would leave a pool of curved oligomers (Cn) in a dead-end. By means of another pathway (Figure 3C), GTP induces the lateral accretion and straightening of previously curved linear tubulin oligomers (Cn) into two-dimensional fragments of microtubule wall (Snm). This second pathway, which is more appealing to us, is similar to the proposed pathway of taxol-induced microtubule nucleation (*35*) and also equivalent to the inverse of the characteristic depolymerization process involving the peeling of coiled protofilaments from a microtubule end (*6*). Lateral oligomer association and straightening are coupled stepwise. Once two curved oligomers make a limited lateral microtubule-like contact (only one monomer from each oligomer), they may either dissociate or cooperatively propagate their contact while straightening the rest of the monomers, in a unimolecular reaction. The latter results in a pair of laterally associated microtubule protofilament segments, that is, a polymerization nucleus. Such a process could operate both for microtubule nucleation in solution, and also at microtubule growing ends, giving rise to smoothly curved elastic sheets later closing into microtubules; it would be compatible with protofilaments having the same intrinsic curvature in growing and shrinking microtubules (*36*). Finally, the shift we propose in the tubulin−GTP straightening energetics, from that previously thought to be favorable to unfavorable, implies that tubulin−GTP is somewhat tensioned within a microtubule (but not metastable as tubulin−GDP), which might be relevant to models of dynamic instability and force production by depolymerizing microtubules.

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SUPPORTING INFORMATION AVAILABLE

Statistics for the superpositions of tubulin and FtsZ (one table); structural superpositions (as PDB files); and a movie of the putative tubulin and FtsZ switch (in PPT and MPEG formats). This material is available free of charge via the Internet at http://pubs.acs.org.

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