

Structural models for the self-assembly and microtubule interactions of γ -, δ - and ϵ -tubulin

Yuki F. Inclán and Eva Nogales*

Howard Hughes Medical Institute

Molecular and Cell Biology Department, University of California at Berkeley, Berkeley, CA 94720-3200, USA

Life Science Division, Lawrence Berkeley National Laboratory, University of California at Berkeley, Berkeley, CA 94720-3200, USA

*Author for correspondence (e-mail: Enogales@lbl.gov)

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SUMMARY

$\alpha\beta$ -tubulin heterodimers self-assemble to form microtubules nucleated by γ -tubulin in the cell. γ -tubulin is believed to recruit the $\alpha\beta$ -tubulin dimers that form the minus ends of microtubules, but the molecular mechanism of this action remains a matter of heated controversy. Still less is known about the function and molecular interactions of δ -tubulin and ϵ -tubulin. δ -tubulin may seed the formation of the C triplet tubules in the basal bodies of *Chlamydomonas* and ϵ -tubulin is known to localize to the centrosome in a cell cycle-dependent manner. Using the structure of $\alpha\beta$ tubulin as a model, we have analyzed the sequences of γ -, δ - and ϵ -tubulin in regions corresponding to different polymerization interfaces in the tubulin $\alpha\beta$ dimer. The sequence comparisons sometimes show clear conservation, pointing to similar types of contacts being functionally important for the new tubulin considered.

Conversely, certain surfaces show marked differences that rule out equivalent interactions for non-microtubular tubulins. This sequence/structure analysis has led us to structural models of how these special tubulins may be involved in protein-protein contacts that affect microtubule self-assembly. δ -tubulin most likely interacts longitudinally with α -tubulin at the minus ends of microtubules, while ϵ -tubulin most likely binds to the plus end of β -tubulin. Conservation of key residues in γ -tubulin suggests that it is capable of longitudinal self-assembly. The implications for the protofilament and template models of nucleation are considered.

Key words: γ -tubulin, δ -tubulin, ϵ -tubulin, Tubulin assembly, Microtubule nucleation, Structural homology

INTRODUCTION

Microtubules are essential cytoskeletal elements involved in chromosome segregation, cell shape, intracellular transport and flagellar beating (Hyams and Lloyd, 1993). Microtubules are composed of $\alpha\beta$ dimers stacked head-to-tail to form protofilaments that aggregate laterally in a parallel fashion to form a cylinder (Fig. 1a) (Amos and Klug, 1974) (for a recent review see Nogales, 2000). The tubulin dimer structure has been determined by electron crystallography of zinc-induced tubulin sheets to 3.7 Å resolution (Nogales et al., 1998b; Fig. 1b,c). A high-resolution model of the microtubule was obtained by docking the tubulin dimer into a 20 Å map of the microtubule obtained by cryo-electron microscopy and helical reconstruction methods (Nogales et al., 1999). The structure and the microtubule model identified amino acid residues involved in inter and intradimer association, and interactions between protofilaments.

Microtubules have a defined polarity consisting of a highly dynamic plus end and a less dynamic centrosome-associated minus end (Euteneuer and McIntosh, 1981). This polarity can be extended to refer to the ends of the dimer and each tubulin monomer. The plus end of each monomer (yellow in Fig. 1d) includes N-terminal domain loops directly involved in nucleotide binding. The minus end (green in Fig. 1d) includes the 'catalytic' region in the second domain of tubulin, which

contacts the nucleotide of the next monomer along the protofilament via longitudinal contacts. Within the $\alpha\beta$ tubulin dimer, the 'minus end' of β -tubulin contacts the plus end of α -tubulin burying the N-site (non-exchangeable site) nucleotide. In contrast polymerization of $\alpha\beta$ dimers into protofilaments results in the burial and hydrolysis of the E-site (exchangeable site) nucleotide (Nogales et al., 1998a) as the minus end of α -tubulin interacts with the plus end of β -tubulin. In the most commonly observed microtubule helical lattice (the B-lattice, 3-start helix, 13 protofilaments) (Song and Mandelkow, 1995), lateral contacts are mostly homologous (α - α , β - β), except at the seam where α - β , β - α contacts occur. Lateral interactions between protofilaments involve the surface containing helix H3 of the $\alpha\beta$ -tubulin dimer (red in Fig. 1d), which contacts the M loop (ML) surface of dimers in the adjacent protofilament (purple in Fig. 1d) (Nogales et al., 1999).

The centrosome, classically described as a pair of centrioles surrounded by pericentriolar material (PCM), serves as the cellular site for microtubule nucleation. Nucleation and anchoring of microtubules is localized to the PCM where the 30S γ -TuRC (γ -tubulin ring complex or gammasome) is found (Murphy et al., 1998; Stearns et al., 1991; Zheng et al., 1995). This ring-shaped complex, (Moritz et al., 1995) comprised of γ -tubulin and up to six other proteins, nucleates microtubules through an unknown mechanism. γ -Tubulin also exists in a smaller complex, the γ -TuSC (first discovered in yeast; Knop

and Schiebel, 1997), consisting of two γ -tubulin subunits and two associated γ -tubulin-binding proteins of approximately 100 kDa each: Spc98p and Spc97p in *S. cerevisiae*. These proteins have homologs across many species including humans and *Xenopus* (Martin et al., 1998; Tassin et al., 1998). γ -TuSCs are found in the cytoplasm, yeast spindle pole body and as minimal components of the γ -TuRC in the centrosome (for a review see Wiese and Zheng, 1999).

Two competing models have been proposed for microtubule nucleation by γ -tubulin. The γ -TuRC, which has approximately the same diameter as the microtubule and in the 'template model' is presumed to contain laterally associated γ -tubulin subunits, nucleates by making longitudinal contacts with $\alpha\beta$ dimers to form the minus end (Zheng et al., 1995). In the 'protofilament model', a ring of longitudinally stacked γ -tubulin subunits unfolds to form a short protofilament that nucleates microtubules via lateral contacts with the $\alpha\beta$ -dimers (Erickson and Stoffler, 1996). Although recent studies using electron tomography and immunoelectron microscopy (Keating and Borisy, 2000; Moritz et al., 2000; Wiese and Zheng, 2000) seem to confirm the template model, new biochemical evidence (Leguy et al., 2000) has been interpreted as supporting a modified protofilament model (Erickson, 2000).

Two new tubulin isoforms have recently been discovered. δ -tubulin was identified from *UNI3* gene mutants missing C microtubules in the basal bodies of *Chlamydomonas* (Dutcher and Trabuco, 1998). Basal bodies at the base of cilia and flagella are structurally similar to centrioles in the centrosome. Both have ninefold symmetry and are formed by triplet microtubules (A, B and C tubules), which make up their outer wall. The *UNI3* mutants had malformed and dysfunctional flagella. Human δ -tubulin was subsequently found in the human genome database, and has 40% sequence similarity to the *Chlamydomonas* δ -tubulin protein (Chang and Stearns, 2000). Immunofluorescence studies show that human δ -tubulin localizes to the centrosome, being more prominent between the centrioles (Chang and Stearns, 2000), and partially co-localizes with centrin and γ -tubulin.

ϵ -tubulin was identified from the human genome database on the basis of sequence similarity to other tubulins (Chang and Stearns, 2000). Like δ -tubulin, ϵ -tubulin does not have a yeast homolog, is distinct from all other tubulins and localizes to the centrosome. Immunofluorescence experiments show that its localization is cell-cycle dependent (Chang and Stearns, 2000). If the cell has only one centrosome, ϵ -tubulin co-localizes with γ -tubulin, but in cells with duplicated centrosomes which replicate semi-conservatively, ϵ -tubulin localizes with only one of the centrosomes whereas γ -tubulin localizes with both. ϵ -tubulin associates with the old centrosome, as shown by analyzing the results with respect to primary cilium, and is subsequently recruited to the new centrosome after duplication. Apparently, the localization of ϵ -tubulin does not affect the ability of the centrosome to nucleate microtubules (Chang and Stearns, 2000).

Little is known about the self-assembly and/or interaction of γ , δ and ϵ -tubulin with $\alpha\beta$ -tubulin and the microtubule. Our approach here has been to use high-resolution structural knowledge of the $\alpha\beta$ -dimer and of tubulin-tubulin interactions in the microtubule to generate a model of how other tubulins may interact with one another. This type of analysis is validated

by a previous comparison of α and β tubulins with the structurally similar bacterial self-assembling protein, FtsZ (Nogales et al., 1998a). FtsZ (for filamenting temperature-sensitive mutant Z) is a GTPase essential for cell division in eubacteria and archaea (de Boer et al., 1992). While FtsZ has very little overall sequence identity to α - and β -tubulin (<15%), this identity occurs at very significant residues (Erickson, 1995). Identical or highly conserved residues are in direct contact with the nucleotide (Nogales et al., 1998a). Given the structural similarity of α - and β -tubulin to FtsZ in spite of limited sequence identity, it is reasonable to assume that the structures of γ -, δ - and ϵ -tubulin can be faithfully approximated using the atomic structure of $\alpha\beta$ -tubulin. We have used the known structure of α - and β -tubulin and sequence similarity with the rarer γ -, δ - and ϵ -tubulins, to investigate whether assembly surfaces are likely to be conserved across different tubulin families.

MATERIALS AND METHODS

The sequences of porcine α and β -tubulin (used for the electron crystallographic atomic model) were compared to human γ , δ , and ϵ -tubulin and *Methanococcus jannaschii* (M. jan) FtsZ. The sequences were initially aligned using ClustalW 1.8 (Thompson et al., 1994) and subsequently manually amended to best fit the structure backbone by restricting gaps and insertions to loop regions (see Fig. 2). Homologies of the entire sequences of the different tubulins were assessed using the Dayhoff substitution matrix (Taylor, 1986). Residues in γ -, δ -, and ϵ -tubulin corresponding to those in α - and β -tubulin involved in polymerization contacts were compared and mapped, using *Insight II* (v2.2.0, Biosym Technologies), onto the α -, β -tubulin crystallographic structures, or onto human γ -tubulin structure obtained by homology modeling (Paluh et al., 2000). The four surfaces closely examined contain residues in $\alpha\beta$ tubulin that are involved in longitudinal dimer contacts (minus end and plus end surfaces) or lateral protofilament contacts (H3 lateral and ML lateral surfaces), as defined in Nogales et al., 1999 (see also Fig. 1d):

The minus end surface, including residues from the H3-S4 loop, T7 loop, H10 helix, and H10-S9 loop.

The plus end surface, including residues from helix H1, strand S3, loop T3, loop T5, helix H6, H6-H7 loop, H11 helix and H11-H12 loop.

The H3 lateral surface, involving loop H1-B2, loop H2-B3, strand S3, helix H3, and the H4-S5 loop.

The ML lateral surface, including helix H6, loop H6-H7, loop S7-H9, helix H9, loop H9-S8 and the H10 helix.

Homologies in these particular surfaces were assessed using a substitution matrix for solvent exposed residues (Bordo and Argos, 1991).

RESULTS

Sequence comparisons

The sequences of porcine α - and β -tubulin, human γ -, δ - and ϵ -tubulin, and M. jan FtsZ were aligned and compared (see Fig. 2). Table 1 shows the overall homology and identity between tubulin families. α - and β -tubulin are more alike than any other pair of tubulins. γ -tubulin has higher similarity to β -tubulin than to α -tubulin, while neither δ - nor ϵ -tubulin shows a preferential similarity to either of the microtubular tubulins. δ -tubulin stands out as the most divergent of the five known tubulins.

Table 2 shows homology and identity concerning microtubule polymerization contacts between tubulin families. γ -tubulin shares considerable sequence similarity with the H3 surfaces of α - and β -tubulin. δ -tubulin has significant similarity to the plus end of α - and β -tubulin and the ML surface of α -tubulin, with the ML surface being the most divergent for all other tubulins, and ϵ -tubulin has strong similarity to the minus ends of α - and β -tubulin.

For all tubulins, highly conserved residues are concentrated in the N-terminal nucleotide binding pocket and in the catalytic region within the second domain. In particular, as was the case for $\alpha\beta$ -tubulins and FtsZ (Nogales et al., 1998a), identical residues across the tubulin families include those directly involved in binding the nucleotide (within loops T1 to T7 and helices H1 and H7). Interestingly, there are exceptions where some of those motifs drastically diverge from the consensus, such as loop T3 for γ -tubulin and loop T7 for δ -tubulin (see later).

We have looked in detail at the level of sequence identity and similarity present in γ -, δ -, and ϵ -tubulin in those regions that correspond to polymerization interfaces in $\alpha\beta$ -tubulin. These regions, indicated in Fig. 1d and described in the Materials and Methods, are: the minus end surface (including loop T7), the plus end surface (including most of the nucleotide binding loops), the H3 lateral surface and the ML lateral surface. Special emphasis has also been put into the identification of sequence insertions and deletions in these and other parts of the sequence.

Table 1. Sequence comparison between tubulin isoforms showing percentages of overall identity and similarity

Compared	% Identical	% Similar
α, β	41	58
α, γ	31	47
β, γ	33	52
α, δ	22	36
β, δ	22	35
α, ϵ	30	45
β, ϵ	30	44
γ, δ	27	43
γ, ϵ	28	44
δ, ϵ	23	36

See Materials and Methods for substitutions used.

Table 2. Comparison of residues located on microtubule polymerization surfaces between α - and β -tubulin, and γ -, δ - and ϵ -tubulin

Longitudinal contacts				Lateral contacts			
Surface compared	Tubulin compared	% Identical	% Similar	Surface compared	Tubulin compared	% Identical	% Similar
α -minus ($n=23$)	γ	26	39	α -H3 side ($n=31$)	γ	35	48
	δ	9	22		δ	29	38
	ϵ	39	48		ϵ	29	48
α -plus end ($n=27$)	γ	22	44	α -ML side ($n=28$)	γ	18	32
	δ	22	63		δ	21	54
	ϵ	19	37		ϵ	7	14
β -minus end ($n=23$)	γ	17	30	β -H3 side ($n=31$)	γ	23	61
	δ	13	22		δ	29	61
	ϵ	43	48		ϵ	26	42
β -plus end ($n=27$)	γ	33	52	β -ML side ($n=28$)	γ	18	32
	δ	30	59		δ	7	18
	ϵ	33	52		ϵ	7	14

n is the number of residues compared.

See Materials and Methods for residues compared and substitutions used.

Positions of insertions and deletions

A dramatic sequence deviation concerning a highly conserved tubulin motif occurs for all four known δ -tubulins in loop T7, further emphasizing divergence of this tubulin with respect to the rest. The consensus sequence GxxNxD is changed to YxxN-P. We expect this divergence to be significant for the function of δ -tubulin, since the consensus GxxNxD is conserved between all other tubulins and FtsZ. Conservation of this region between tubulins and FtsZs led to the proposition that longitudinal interactions between FtsZ subunits involve identical structural elements to those in tubulin (Nogales et al., 1998a), a view that was later confirmed by electron microscopy and image reconstruction (Löwe and Amos, 1999).

A striking characteristic of the γ -tubulin sequence is the Gly-Gly insertion in the T3 loop (plus end surface), which precedes a highly conserved sequence within the tubulin family (GNNWAXG). This insertion and a point deletion immediately following the consensus region are consistent throughout most sequenced γ -tubulins (data not shown). Interestingly, this position is also the location of the only large insertion in M. jan FtsZ with respect to $\alpha\beta$ -tubulins (Nogales et al., 1998a; see arrow in Fig. 2).

The loop between B9-B10, which faces the lumen of the microtubule in $\alpha\beta$ -tubulin, includes an eight residue insertion in the α -tubulin subunit, which occludes the site that is occupied by taxol in β -tubulin (Nogales et al., 1998b). Both γ - and δ -tubulin have shorter insertions in this loop of three and two amino acids, respectively, whereas ϵ -tubulin, like β -tubulin, does not have an insertion here. The poorly ordered loop between H1 and B2, which mostly faces the lumen of the microtubule and curves toward the H3 surface, is believed to be involved in lateral contacts between protofilaments for $\alpha\beta$ -tubulin and is generally not conserved among the tubulin family. Finally, the acidic C-terminal tail characteristic of $\alpha\beta$ -tubulin has lost its acidic character in γ - and ϵ -tubulin, and is missing altogether in δ -tubulin. This tail is disordered in the crystal structure of $\alpha\beta$ -tubulin, but expected to be on the outside surface of the microtubule (Nogales et al., 1999). These C-terminal residues have been implicated in the binding of basic microtubule-associated proteins (MAPs) (for a review see Sackett, 1995), and they seem to be essential for the function of monomeric kinesins (for a recent result see

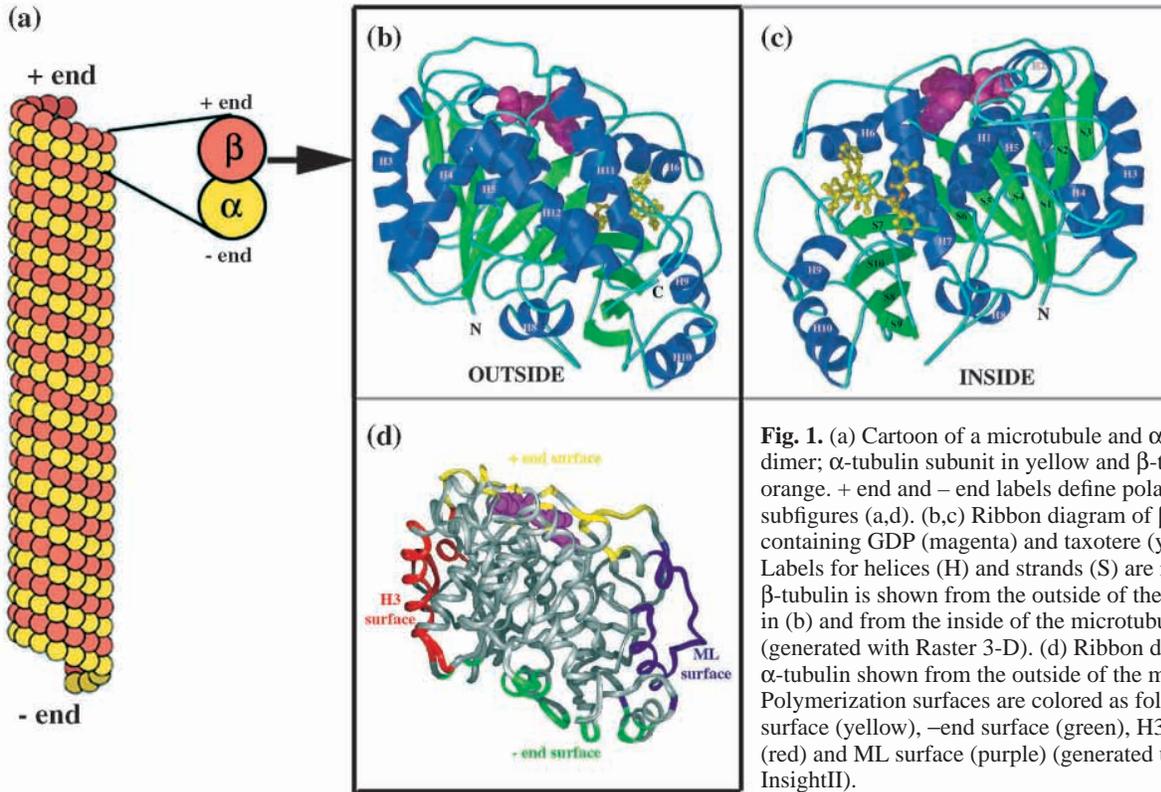


Fig. 1. (a) Cartoon of a microtubule and $\alpha\beta$ -tubulin dimer; α -tubulin subunit in yellow and β -tubulin in orange. + end and - end labels define polarity for all subfigures (a,d). (b,c) Ribbon diagram of β -tubulin containing GDP (magenta) and taxotere (yellow). Labels for helices (H) and strands (S) are included. β -tubulin is shown from the outside of the microtubule in (b) and from the inside of the microtubule in (c) (generated with Raster 3-D). (d) Ribbon diagram of α -tubulin shown from the outside of the microtubule. Polymerization surfaces are colored as follows: +end surface (yellow), -end surface (green), H3 surface (red) and ML surface (purple) (generated using InsightII).

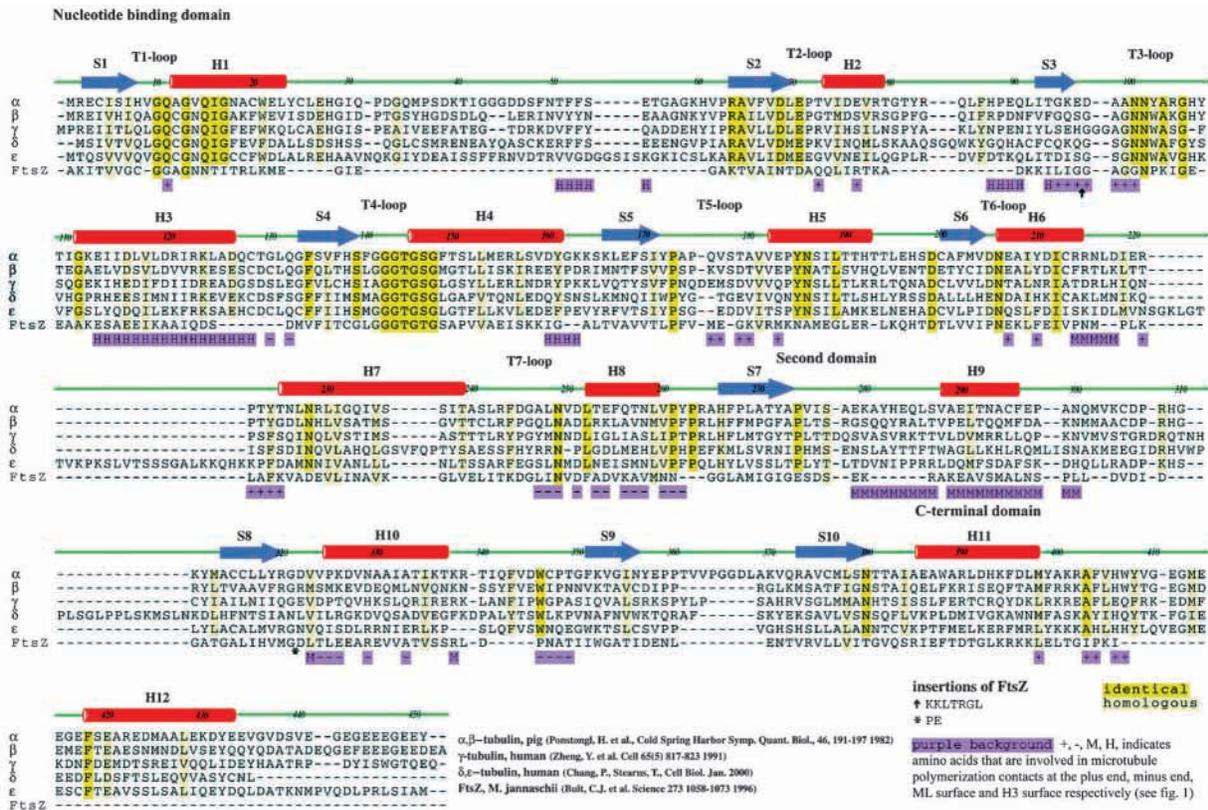


Fig. 2. Sequence alignment of pig α - and β -tubulin, human γ -, δ - and ϵ -tubulin and M. Jan FtsZ. Secondary structural elements are indicated and labeled as for Fig 1b.c. T1 to T7 indicate loops directly involved in nucleotide contacts (from Nogales et al., 1998b). Yellow boxes indicate sequence identity and beige boxes indicate similarity. A minimum of five residues are identical or similar in each boxed column. The position of the arrow and the star indicate insertions of FtsZ. Underlying purple bars mark the residues that are involved in tubulin-tubulin contacts. These residues are used throughout the paper in the comparisons of the different tubulin families.

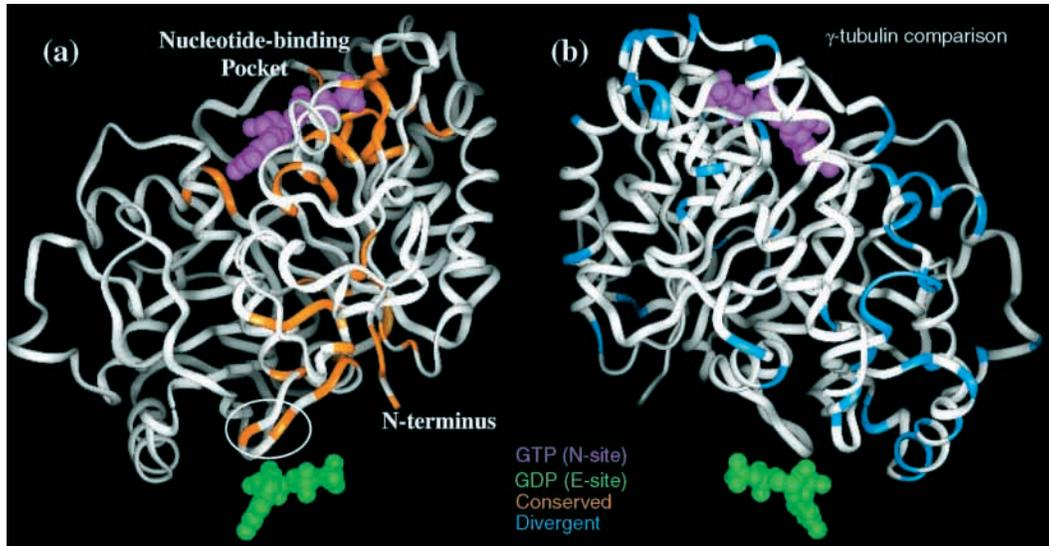


Fig. 3. (a) Homology model of human γ -tubulin (Paluh et al., 2000), with the nucleotide shown in magenta for this subunit. An additional nucleotide is shown in green that would correspond to another tubulin subunit making longitudinal contacts. (a) Orange indicates the position of residues conserved between pig α - and β -tubulin and human γ -tubulin. The view corresponds to the inside of the microtubule. (b) Blue indicates the location of residues conserved between pig α - and β -tubulin but clearly divergent in human γ -tubulin. The tubulin monomer is shown from the outside of the microtubule. Figure generated with InsightII.

Kikkawa et al., 2000). The dramatic divergence of γ -, δ - and ϵ -tubulin with respect to this region strongly suggests that these proteins will not interact with classical MAPs or motor proteins that rely on an electrostatic attachment to the C terminus. However, it should be noted that helices H11 and H12 are still present in these divergent tubulins (in contrast with their absence in FtsZ), indicating that they may have maintained some binding surfaces for conventional motors.

γ -Tubulin analysis

Totally conserved residues between α -, β - and γ -tubulin are located in regions that directly interact with the N-terminal residues, in both the nucleotide-binding pocket and the catalytic region (T7, H8) located across longitudinal contacts (Fig. 3a, orange). In addition, γ -tubulin shares considerable sequence similarity with the H3 surfaces of α - and β -tubulin and the plus end of β -tubulin (see Table 2 and Fig. 4).

Those residues that are conserved between α and β -tubulin but are totally divergent in γ -tubulin are concentrated on one surface of the protein that corresponds to a groove between protofilaments on the outside of the microtubule (see Fig. 3b, blue). This region includes the H9-B8 loop, a site of insertions of

variable lengths for different γ -tubulins. Furthermore, this is a region where plant γ -tubulin drastically diverges from mammalian γ -tubulins (B. Oakley, personal communication). Thus, this site has most likely changed to accommodate specific protein-protein interactions of γ -tubulin, possibly with

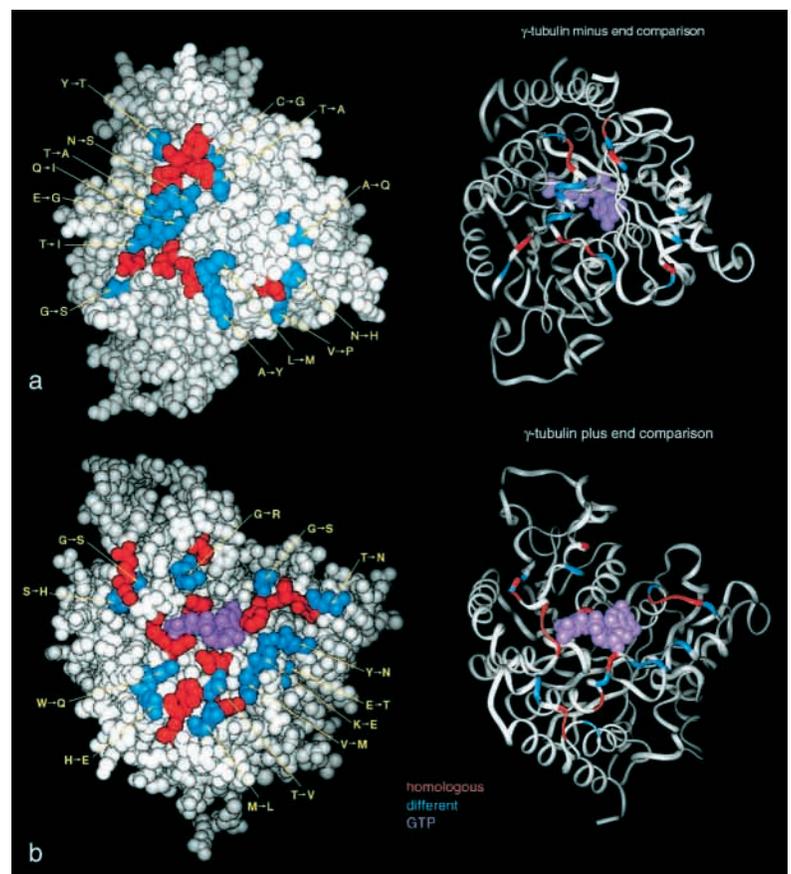


Fig. 4. γ -tubulin minus end compared with α -tubulin (a), and γ -tubulin plus end compared with β -tubulin (b). Both space-filled model (left) and ribbon diagram (right) show the residues expected to be involved in longitudinal contacts; identical or homologous residues are shown in red, different residues are shown in light blue. The nucleotide is shown in purple. Yellow arrows and labels on the space-filled model indicate residue substitutions made from α -tubulin (a) and β -tubulin (b). Figure generated with InsightII.

other proteins in the γ -TuRC, that change across species (much as the C terminus tail does for α and β tubulins).

In addition, most γ -tubulin sequences have insertions in two nucleotide-binding loops: two extra Gly in T3 (mentioned previously), and an insertion in T5 that is commonly an Asp or Glu. Loop T5, involved both in nucleotide binding and longitudinal contacts between subunits, is a site of variability across tubulin families, with δ -, ϵ -tubulin and FtsZ, having a one residue deletion in this loop with respect to α - and β -tubulin. This diversity may give rise to longitudinal contacts of variable strength and/or geometry between the different tubulins. Interestingly, the proposed catalytic residue in α -tubulin, Glu254 (a conserved Lys in β -tubulin), is either Gly or Ser in γ -tubulins (except in the case of *C. elegans* and *Guillardia theta*, which have very divergent sequences in this region and contain a deletion with respect to other γ -tubulins). The lack of acidic residues at this position strongly suggests that if γ -tubulin could make longitudinal contacts with other tubulin molecules at its minus end, the contact would not bring about the hydrolysis of the nucleotides in the contacting tubulin. Furthermore, given the conservation of other residues involved in longitudinal contacts on this surface of γ -tubulin, longitudinal contacts on its minus ends are most likely (e.g. in the form of a γ -tubulin dimer, see later). Finally, some γ -tubulins also have an Asp inserted in the M loop, a region directly involved in lateral contacts between tubulins.

δ -Tubulin analysis

Totally conserved residues between α -, β - and δ -tubulin are located mainly in the N-terminal nucleotide-binding pocket. δ -tubulin exhibits quite low sequence similarity to the minus ends of both α - and β -tubulin. Instead, its most significant sequence similarity occurs at the plus end of α - and β -tubulin (see Fig. 5) and the ML surface of α -tubulin.

All four currently sequenced δ -tubulins have at least a three amino acid insertion in the H2-B3 loop, possibly involved in lateral contacts in the microtubule, and a minimum of five amino acids inserted in core helix H7. Most significantly, a 14 amino acid insertion in the H9-B8 loop is very conserved within the δ -tubulin subfamily. This loop is on the outside surface of the $\alpha\beta$ -dimer close to the M-loop, which is involved in lateral contacts. It defines a pocket in the tubulin surface that, as proposed for γ -tubulin above, could be used to make protein-protein contacts specific for this type of tubulin. Finally, the residue corresponding to the catalytic α :Glu254 is a homologous Asp or Glu in three out of the four known δ -tubulins, but diverges to an Ala in *Trypanosoma brucei* δ -tubulin (Vaughan et al., 2000).

ϵ -Tubulin analysis

Like γ - and δ -tubulin, totally conserved residues between α -, β - and ϵ -tubulin are located in the N-terminal nucleotide-binding region. Other conserved residues are spread throughout the structure, with the most divergent regions corresponding to the ML and H10.

In contrast with γ - and δ -tubulin, ϵ -tubulin has strong similarity to the minus ends of α - and β -tubulin (Fig. 6), which suggests an interaction with the plus end of the microtubule. The corresponding amino acid residue to the catalytic α :Glu254 is Glu in humans as well as in *T. brucei* ϵ -tubulin. ϵ -Tubulin also has considerable homology to the H3 surfaces

of α - and β -tubulin. With respect to other tubulins it has a 5 amino acid insertion in the flexible H1-B2 loop and a 26 amino acid insertion in the H6-H7 loop. Both of these insertions map to the plus end of α - and β -tubulin towards the lumen of the microtubule and are also present in *T. brucei* ϵ -tubulin, the only other ϵ -tubulin known thus far (Vaughan et al., 2000). The large 26 residue insertion is reminiscent of the second domain in GAPDH. GAPDH has a nucleotide-binding domain with the same topology as tubulin, and a C-terminal helix after the second domain, which lines up with the core helix, H7, in tubulin (Nogales et al., 1998a). This large difference in the plus end should make any interaction of ϵ -tubulin with the minus end of the microtubule very different from that of any other tubulin.

DISCUSSION

The overall sequence identity/similarity of γ -, δ - and ϵ -tubulins to the α - and β -tubulins (20-30% identical, 35-45% similar) is high enough to assume that the overall structure of all tubulins will be shared. This assumption is strongly supported by the striking structural similarity between FtsZ, α - and β -tubulin (Nogales et al., 1998a), in spite of modest sequence identity between these proteins (~15% identical). Furthermore, this common topology, and the conservation of landmark residues involved in nucleotide interaction and longitudinal contacts in the latter, was sufficient to predict polymerization surfaces in FtsZ filaments (Löwe and Amos, 1999; Nogales et al., 1998a). The experience with this distant tubulin relative supports the use of sequence conservation as a tool to predict self-assembly of the new tubulins and their possible interactions with microtubules.

Nucleotide-binding and catalytic domains

Regions in the tubulin sequences completely conserved across families are those involved in nucleotide binding. This is not surprising considering similar conservation is also present between α -, β -tubulin and FtsZ. The T3 loop is involved in binding the γ -phosphate in the nucleotide. In γ -tubulin part of this loop is significantly altered by the presence of an insertion reminiscent of that in FtsZ. The T3 loop has been proposed to be equivalent to the switch II region in classical GTPases and to serve to propagate a hydrolysis-induced conformational change to H3, consequently affecting lateral contacts (Nogales et al., 1999). The variation in the length of this loop for γ -tubulin and FtsZ with respect to $\alpha\beta$ -tubulin may correspond to a different conformational effect upon loss of the γ -phosphate.

Loop T5 is involved both in nucleotide-ribose binding and longitudinal contacts along protofilaments, and is another region of variable sequence and length among tubulins. We expect these variations to have a more significant effect on longitudinal interactions between tubulin subunits than on nucleotide binding properties. T5 extends out of the plus end of one monomer to make longitudinal contacts with the minus end of the adjacent monomer near the center of the binding surface. Thus, deviations in the T5 loop could affect longitudinal contacts by changing the tubulin-tubulin energy of interaction and/or geometry of the interaction with regard to $\alpha\beta$ -tubulin.

The GxxNx_D consensus sequence in T7, previously

mentioned, comes into close contact with the phosphates of the nucleotide in the following tubulin subunit along a protofilament (Nogales et al., 1998a). This motif is a marker of longitudinal interactions between tubulins. It is absolutely conserved in all tubulin sequences and FtsZ, but greatly diverges in δ -tubulin. This suggests that δ -tubulin is not capable of longitudinal self-assembly, and that any longitudinal interactions with other tubulins must be limited to the minus end (involving only the plus end of the δ -tubulin molecule).

Closely following the conserved GxxNxD sequence is a residue that has been identified in α -tubulin as essential for the hydrolysis of the E-site GTP in β -tubulin. This totally conserved residue, α :Glu254, is positioned close to the γ -phosphate of the E-site nucleotide upon formation of longitudinal contacts between $\alpha\beta$ -tubulin dimers. The equivalent conserved residue in FtsZ is an Asp that, upon mutation to Ala, renders FtsZ incapable of hydrolysis (Dai et al., 1994). In β -tubulin the conserved residue is a Lys that contributes to the monomer-monomer binding within the $\alpha\beta$ -dimer by interacting with the negatively charged phosphates in the N-site nucleotide. For γ -tubulin, the corresponding residue is commonly a Gly or Ser and never acidic. Thus, if γ -tubulin makes contact with other tubulins via its minus end surface, it would most likely be incapable of inducing nucleotide hydrolysis in the adjacent subunit. The equivalent residues in δ - and ϵ -tubulin are an Asp and Glu respectively, thus suggesting GTP hydrolysis activity from these tubulins if they contact other tubulin subunits via their minus ends.

Longitudinal polymerization contacts

Longitudinal interaction of γ -tubulin with α -tubulin at the microtubule minus end

γ -Tubulin has high homology to β -tubulin in the plus end surface involved in longitudinal contacts between dimers. This suggests that it mimics the behavior of β -tubulin by making contact with the minus end of α -tubulin. This conclusion is drawn purely on the basis of sequence similarity and generally agrees with the function of γ -tubulin as a nucleator at the minus end of the microtubule. The two competing models of nucleation, the template model and the protofilament model, both include longitudinal contacts of the plus end surface of γ -tubulin with the minus end surface of α -tubulin (about 13 such contacts will occur in the template model, while only one will occur in the protofilament model).

γ -Tubulin is likely to make longitudinal contacts via its minus end

By the same argument used to predict longitudinal self-assembly of FtsZ, conservation of the GxxNxD sequence in the T7 loop, as well as other regions involved in nucleotide contact, suggests that γ -tubulin subunits self-assemble longitudinally (an interaction of γ -tubulin with the plus end of a microtubule seems less likely). Whether longitudinal contacts would be restricted to a dimer or extend into a protofilament-like structure is not clear. An important point to consider is significant discrepancies in other areas of the minus end surface of γ -tubulin. The HWY motif between the C-terminal helices common to α - and β -tubulin is central to both intra and inter-dimer monomer contacts. The complete loss of this motif in γ -tubulin suggests that a γ -tubulin protofilament would have a geometry/curvature that is significantly different from that in

$\alpha\beta$ -tubulin protofilaments. Additionally, the idea of a γ -tubulin dimer is consistent with the existence of the small γ -TuRC containing two γ subunits and one copy of both Spc97 and Spc98 (or non-yeast equivalents). The Spc proteins may stabilize a single γ - γ longitudinal contact, possibly by contacting a surface formed by residues divergent from α - and β -tubulin (i.e. the H9-B8 loop region). While the idea of a head-to-tail dimer of γ -tubulin forming part of the small complex seems reasonable, it is more problematic to make it compatible with the known approximate diameter and the estimated molecular weight of the large γ -TuRC. The diameter and ~13-fold repeat of the γ -TuRC will require ~13 small complexes, where the γ -tubulin dimers would be interacting laterally in a ring to form the microtubule template. The minimum size of such a structure would be 3.5 MDa, considerably larger than the 2-2.5 MDa estimated by STEM (Y. Zheng, personal communication).

Interaction of δ -tubulin with α -tubulin at the microtubule minus end

The plus end surface of δ -tubulin has significant sequence similarity to α - and β -tubulin, especially with the latter, suggesting it contacts the minus end of α -tubulin. In contrast to γ -tubulin, the minus end of δ -tubulin includes multiple insertions and deletions giving it considerable diverging character from α - and β -tubulin. In particular, δ -tubulin is the only tubulin isoform (along with FtsZ) that has lost the conserved GxxNxD motif. This strongly suggests that it is incapable of making tubulin-tubulin contacts via its minus end in the same manner as α - and β -tubulin. However, divergent surfaces such as this are possible candidates for binding other centrosomal/basal body-associated proteins.

Interaction of ϵ -tubulin with β -tubulin at the microtubule plus end

The most surprising result of our structural analysis is the high sequence identity/similarity of the minus end of ϵ -tubulin with that of α -tubulin. This finding suggests that ϵ -tubulin contacts the plus end of β -tubulin and, accordingly, adds onto a microtubule plus end. This is most intriguing given the localization of ϵ -tubulin to the centrosome. On the opposite surface, ϵ -tubulin has a 26 amino acid insertion in the H6-H7 loop that should greatly change the topology of this surface with respect to α - and β -tubulin. This insertion could prevent ϵ -tubulin from interacting with an incoming dimer or drastically change the geometry of the contact. Thus, our analysis suggests that ϵ -tubulin is an excellent candidate for a plus-end capping protein. The finite centriole microtubules could be good candidates for the location of ϵ -tubulin.

Lateral polymerization contacts

The surfaces involved in longitudinal contacts along $\alpha\beta$ -tubulin protofilaments have greater sequence similarity between α - and β -tubulin than those involved in lateral contacts. This is consistent throughout the tubulin family and gives lateral interactions a special character. A likely role of the variability in these surfaces would be to tune microtubule stability (a large number of sequence changes in tubulins from cold-adapted fish are localized at lateral contacts, specifically in the ML (Detrich et al., 2000)). Additionally, while longitudinal contacts are more specific and tight, lateral

Fig. 5. δ -tubulin plus end surface compared with β -tubulin. The locations of residues involved in longitudinal contacts are mapped on the β -tubulin structure. Both space-filled model (left) and ribbon diagram (right) display identical views and show identical/homologous residues in red and different residues in light blue. GDP is light purple and taxol is pink. Yellow arrows and labels on the space-filled model indicate positions and substitutions made from β -tubulin to δ -tubulin. Models generated with InsightII.

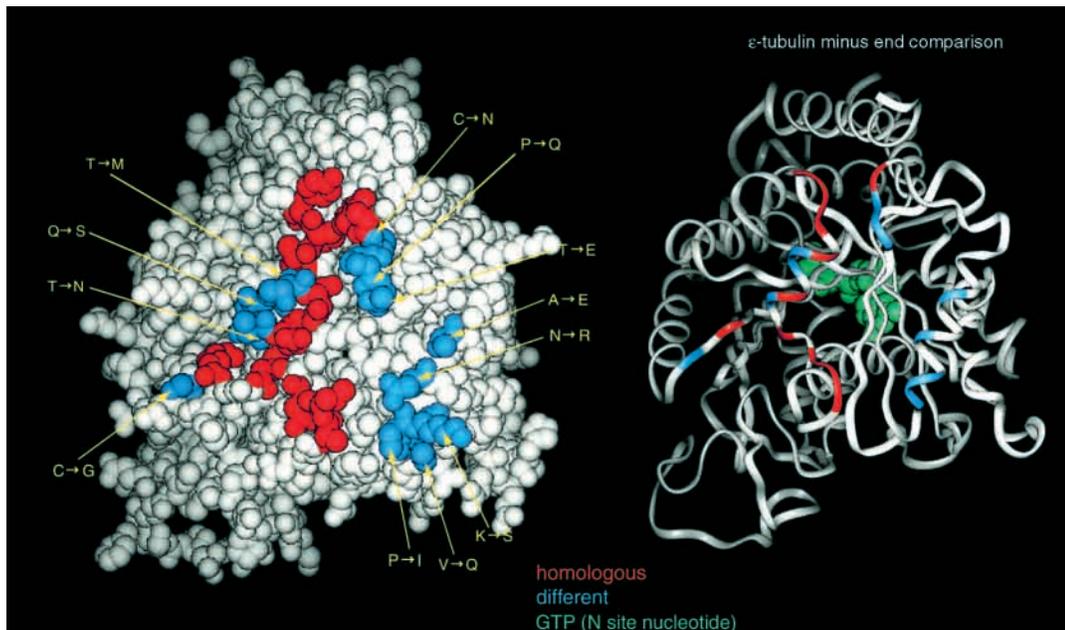
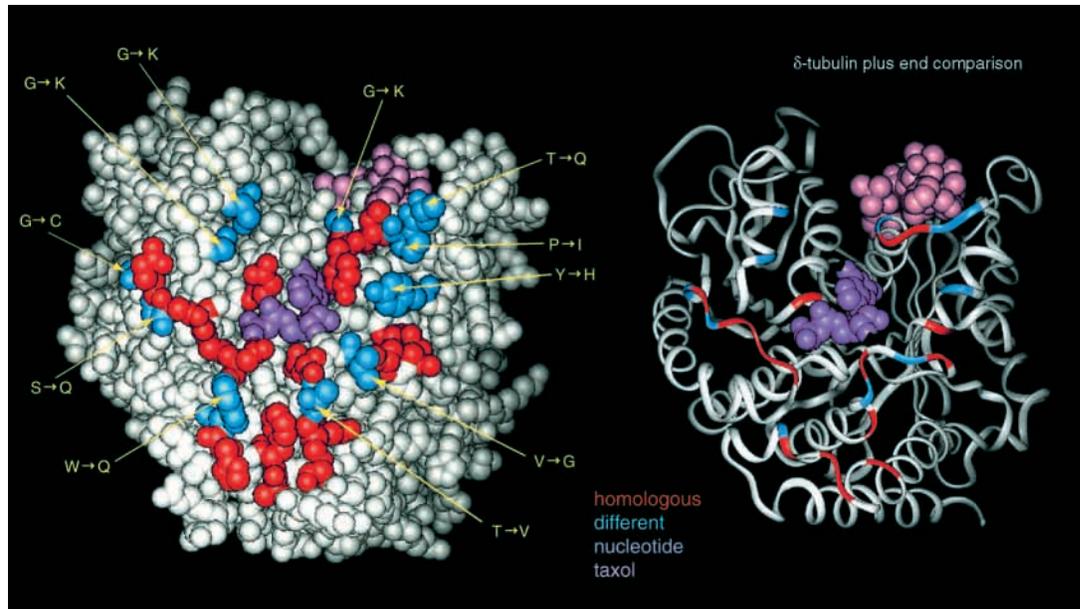


Fig. 6. ϵ -tubulin minus end compared with α -tubulin. The locations of residues involved in longitudinal contacts are mapped on the α -tubulin structure. Both space-filled model (left) and ribbon diagram (right) display identical views and indicate identical/homologous residues (red) and different residues (blue). The ribbon diagram displays GTP in green. Yellow arrows and labels on the space-filled model indicate positions and substitutions made from α -tubulin to ϵ -tubulin. Models generated with InsightII.

contacts have less specificity (as testified by the existence of a microtubule seam) and are more flexible (as indicated by the existence of microtubules composed of different numbers of protofilaments). The general lack of conservation regarding lateral surfaces, even within a certain tubulin family (an example is the highly variable ML), makes extrapolations on prospective lateral contacts in the new tubulin families more difficult. The most likely scenarios for lateral contacts based on our analysis are as follows.

The H3 lateral surface of γ -, δ - and ϵ -tubulin all have similar degrees of homology to α - and β -tubulin

This homology is more significant between γ -tubulin and δ - and β -tubulin, which mildly suggests γ - and δ -tubulin contact the ML surface of α -tubulin. A modified protofilament model of γ -tubulin nucleation activity has been proposed based on the

findings of Carrier and collaborators on the nucleating activity of monomeric γ -tubulin (Erickson, 2000; Leguy et al., 2000). In this model, a single γ -tubulin caps the minus end by making one longitudinal interaction with α -tubulin and one strong lateral interaction with β -tubulin. The lateral interaction involves the H3 lateral surface of γ -tubulin and the ML surface of β -tubulin. Our sequence comparison is compatible with this model, as well as with a template model in which the lateral contacts occur between γ -tubulin subunits.

The ML surface of δ -tubulin has considerable homology to the ML surface of α -tubulin but not with the ML surface of β -tubulin

It should be noted that the ML surface is generally the most divergent segment between tubulins and therefore this homology is of special significance. It indicates a likely lateral

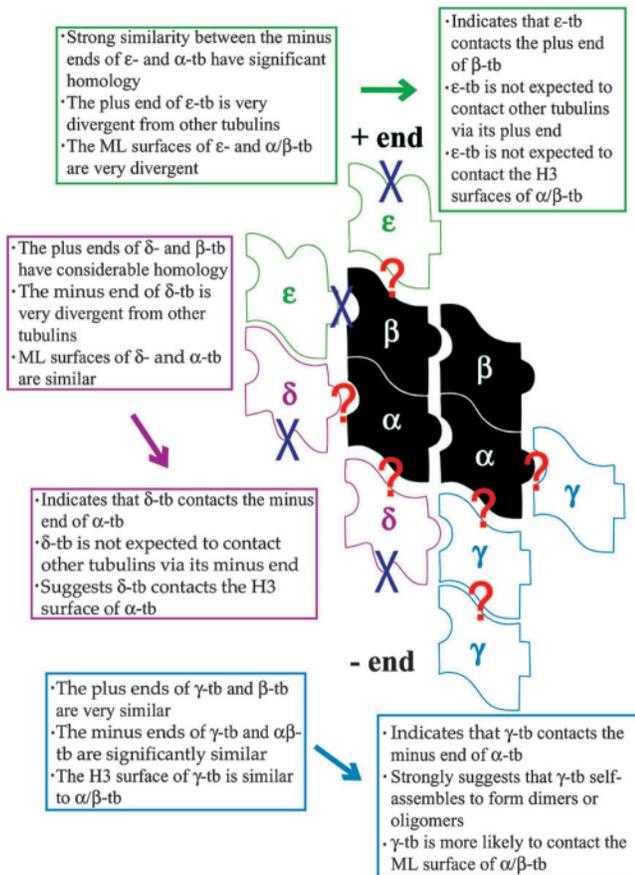


Fig. 7. Cartoon model of possible interactions for γ -, δ - and ϵ -tubulin with $\alpha\beta$ -tubulin. The red question marks indicate a likely interaction whereas the blue Xs indicate a most unlikely interaction. ϵ -tubulin is likely to contact the plus end of β -tubulin because of significant homology with the minus end of α -tubulin. However, the plus end of ϵ -tubulin is very divergent from $\alpha\beta$ -tubulin and it is not expected to interact with other tubulins. The ML surface of ϵ -tubulin and $\alpha\beta$ -tubulin are also very divergent so ϵ -tubulin is not expected to contact the H3 surfaces of α/β -tubulin. δ -tubulin is likely to contact the minus end of α -tubulin because of considerable homology to the plus end of β -tubulin. The ML surface of δ -tubulin is similar to α -tubulin and thus is likely to contact the H3 surface of α -tubulin. The minus end of δ -tubulin is very divergent and not expected to interact with other tubulins. γ -tubulin is likely to contact the minus end of α -tubulin because of considerable homology with the plus end of β -tubulin. γ -tubulin also has significant conservation with the minus end of $\alpha\beta$ -tubulin, suggesting that γ -tubulin is capable of self-assembly to form dimers or oligomers.

contact between the ML surface of δ -tubulin and the H3 surface of α -tubulin. In addition, the ML surface of ϵ -tubulin is significantly divergent from both α - and β -tubulin, indicating that the ML side of ϵ -tubulin contacts neither of these tubulins at their H3 surface.

Conclusions

Based on our sequence/structure analysis of γ -, δ - and ϵ -tubulin, we have generated a model of interactions between these non-microtubular proteins, and α - and β -tubulin (see Fig. 7). δ -tubulin most likely contacts α -tubulin at the microtubule minus end via longitudinal contacts involving its nucleotide

binding surface. The opposite surface of δ -tubulin is not expected to interact with other tubulins because of various insertions and deletions in this area. The situation is reversed for ϵ -tubulin. The 'catalytic' end of ϵ -tubulin is likely to contact β -tubulin at the plus end of the microtubule, while its nucleotide-binding surface is structurally modified by insertions and deletions that render it incapable of interacting with other tubulins via an $\alpha\beta$ -tubulin-like contact. γ -Tubulin is likely to contact the minus end of α -tubulin and may associate laterally with α - or β -tubulin. While not discriminating, these conclusions lend support to the template theory of nucleation. However, conservation of residues involved in longitudinal contacts at the minus end of the γ -tubulin itself, strongly suggest that this tubulin isoform should be capable of self-assembly to form either a dimer or a protofilament-like oligomer.

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REFERENCES

- Amos, L. A. and Klug, A. (1974). Arrangement of subunits in flagellar microtubules. *J. Cell Sci.* **14**, 523-549.
- Bordo, D. and Argos, P. (1991). Suggestions for 'safe' residue substitutions in site-directed mutagenesis. *J. Mol. Biol.* **217**, 721-729.
- Chang, P. and Stearns, T. (2000). Delta-tubulin and epsilon-tubulin: two new human centrosomal tubulins reveal new aspects of centrosome structure and function. *Nature Cell Biol.* **2**, 30-35.
- Dai, K., Mukherjee, A., Xu, Y. and Lutkenhaus, J. (1994). Mutations in FtsZ that confer resistance to SulA affect the interaction of FtsZ with GTP. *J. Bacteriol.* **175**, 130-136.
- de Boer, P., Crossley, R. and Rothfield, L. (1992). The essential bacteria cell-division protein FtsZ is a GTPase. *Nature* **359**, 254-256.
- Detrich, H. W. 3rd, Parker, S. K., Williams, R. G. Jr., Nogales, E. and Downing, K. H. (2000). Cold adaptation of microtubule assembly and dynamics, structural integration of primary sequence changes present in the alpha- and beta-tubulins of Antarctic fishes. *J. Biol. Chem.* **275**, 37038-37047.
- Dutcher, S. K. and Traubco, E. C. (1998). The UNI3 gene is required for assembly of basal bodies of Chlamydomonas and encodes delta-tubulin, a new member of the tubulin superfamily. *Mol. Biol. Cell* **9**, 1293-308.
- Erickson, H. P. (1995). FtsZ, a prokariotic homolog of tubulin? *Cell* **80**, 367-370.
- Erickson, H. P. (2000). γ -tubulin nucleation: template or protofilament. *Nature Cell Biol.* **2**, E93-E96.
- Erickson, H. P. and Stoffer, D. (1996). Protofilaments and rings, two conformations of the tubulin family conserved from bacterial FtsZ to $\alpha\beta$ and γ tubulin. *J. Cell Biol.* **135**, 5-8.
- Euteneuer, U. and McIntosh, J. R. (1981). Polarity of some motility-related microtubules. *Proc. Natl. Acad. Sci. USA* **78**, 372-376.
- Hyams, J. S. and Lloyd, C. W. (1993). *Microtubules*. New York: Wiley-Liss.
- Keating, T. J. and Borisy, G. G. (2000). Immunostuctural evidence for the template mechanism of microtubule nucleation. *Nat. Cell Biol.* **2**, 352-357.
- Kikkawa, M., Okada, Y. and Hirokawa, N. (2000). 15 Å resolution model of the monomeric kinesin motor, KIF1A. *Cell* **100**, 241-252.
- Knop, M. and Schiebel, E. (1997). Spc98p and Spc97p of the yeast gamma-tubulin complex mediate binding to the spindle pole body via their interaction with Spc110p. *EMBO J.* **16**, 6985-6995.
- Leguy, R., Melki, R., Pantaloni, D. and Carlier, M.-F. (2000). Monomeric γ -tubulin nucleates microtubules. *J. Biol. Chem.* **275**, 21975-21980.
- Löwe, J. and Amos, L. A. (1999). Tubulin-like protofilaments in Ca²⁺-induced FtsZ sheets. *EMBO J.* **18**, 2364-2371.
- Martin, O. C., Gunawardane, R. N., Iwamatsu, A. and Zheng, Y. X. (1998). Xgrip109: A gamma tubulin-associated protein with an essential role in gamma tubulin ring complex (gamma TuRC) assembly and centrosome function. *J. Cell Biol.* **141**, 675-687.
- Moritz, M., Braunfeld, M. B., Guénebaud, V., Heuser, J. and Agard, D. A.

- (2000). Structure of the γ -tubulin ring complex: a template for microtubule nucleation. *Nat. Cell Biol.* **2**, 365-370.
- Moritz, M., Braunfeld, M. B., Sedat, J. W., Alberts, B. and Agard, D. A.** (1995). Microtubule nucleation by gamma-tubulin-containing rings in the centrosome. *Nature* **378**, 638-640.
- Murphy, S. M., Urbani, L. and Stearns, T.** (1998). The mammalian γ -tubulin complex contains homologues of the yeast spindle pole body components Spc97p and Spc98p. *J. Cell Biol.* **141**, 663-674.
- Nogales, E., Downing, K. H., Amos, L. A. and Löwe, J.** (1998a). Tubulin and FtsZ form a distinct family of GTPases. *Nat. Struct. Biol.* **5**, 451-458.
- Nogales, E., Wolf, S. G. and Downing, K. H.** (1998b). Structure of the $\alpha\beta$ tubulin dimer by electron crystallography. *Nature* **391**, 199-203.
- Nogales, E., Whittaker, M., Milligan, R. A. and Downing, K. H.** (1999). High resolution structure of the microtubule. *Cell* **96**, 79-88.
- Nogales, E.** (2000). Structural insights into microtubule function. *Annu. Rev. Biochem.* **69**, 277-302.
- Paluh, J. L., Nogales, E., Oakley, B. R., McDonald, K., Pidoux, A. L. and Cande, Z.** (2000). A mutation in γ -tubulin alters microtubule dynamics and organization and is synthetically lethal with the kinesin-like protein Pkl1p. *Mol. Biol. Cell* **11**, 1225-1239.
- Sackett, D. L.** (1995). Structure and function in the tubulin dimer and the role of the acidic carboxyl terminus. In *Proteins: Structure, Function, and Engineering* Vol. 24 (ed. B. B. Biswas and S. Roy), pp. 255-302. New York: Plenum Press.
- Song, Y. H. and Mandelkow, E.** (1995). The anatomy of flagellar microtubules: polarity, seams, junctions, and lattice. *J. Cell Biol.* **128**, 81-94.
- Stearns, T., Evans, L. and Kirschner, M.** (1991). Gamma-tubulin is a highly conserved component of the centrosome. *Cell* **65**, 825-836.
- Tassin, A. M., Celati, C., Moudjou, M. and Bornens, M.** (1998). Characterization of the human homologue of the yeast Spc98p and its association with gamma-tubulin. *J. Cell Biol.* **141**, 689-701.
- Taylor, W. R.** (1986). The classification of amino acid conservation. *J. Theor. Biol.* **119**, 205-218.
- Thompson, J. D., Higgins, D. G. and Gibson, T. J.** (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673-4680.
- Vaughan, S., Attwood, T., Navarro, M., Scott, V., McKean, P. and Gull, K.** (2000). New tubulins in protozoal parasites. *Curr. Biol.* **10**, R258-R259.
- Wiese, C. and Zheng, Y.** (1999). Gamma-tubulin complexes and their interaction with microtubule-organizing centers. *Curr. Opin. Struct. Biol.* **9**, 250-259.
- Wiese, C. and Zheng, Y.** (2000). A new function for the γ -tubulin ring complex as a microtubule minus-end cap. *Nat. Cell Biol.* **2**, 358-364.
- Zheng, Y., Wong, M. L., Alberts, B. and Mitchison, T.** (1995). Nucleation of microtubule assembly by a γ -tubulin-containing ring complex. *Nature* **378**, 578-583.