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COMMENTARY

Analysis of the γ-tubulin sequences: implications for the functional properties of γ-tubulin

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INTRODUCTION

γ-Tubulin was initially identified as a suppressor of an *Aspergillus* β-tubulin mutation (Oakley and Oakley, 1989). Highly homologous genes have since been identified in a variety of other eucaryotes (see Tables 1 and 2), and immunofluorescence studies have confirmed that γ-tubulin is expressed in a wide variety of eucaryotic cells. These observations indicate that γ-tubulin is functionally important, and this has been confirmed by mutational analysis (Oakley et al., 1990; Horio and Oakley, 1994) and by the elegant demonstration that the human γ-tubulin gene product supports the growth and division of *Schizosaccharomyces pombe* (Horio and Oakley, 1994).

γ-TUBULIN DISTRIBUTION, RECRUITMENT, AND ROLE IN MICROTUBULE NUCLEATION

γ-Tubulin is specifically concentrated at microtubule organizing centers (MTOCs), including the highly structured spindle pole bodies of fungi (Oakley et al., 1990; Horio et al., 1991), the basal bodies of ciliated epithelia (Muresan et al., 1993), the centrosomes of animal tissue culture cells and neurons (Zheng et al., 1991; Stearns et al., 1991; Joshi et al., 1992; Baas and Joshi, 1992; Gueth-Hallonet et al., 1993; Lajoie-Mazenc et al., 1994), and the dispersed MTOCs of plant cells (McDonald et al., 1993; Liu et al., 1993, 1994). A primary location of γ-tubulin is therefore towards the (–)ends of microtubules. Immunogold labelling does however indicate that some γ-tubulin can be dispersed along the length of individual microtubules in both plant and animal cells (Liu et al., 1993; Lajoie-Mazenc et al., 1994; Hoffman et al., 1994). The relevance of this secondary localization is unclear, since the method cannot readily distinguish between functional γ-tubulin, a storage form of γ-tubulin (see below), and γ-tubulin in the process of being redistributed. For example, the formation of a novel MTOC is involved in the assembly of the microtubules forming the mid-bodies of animal cells and the phragmoplast of plant cells (Julian et al., 1993; Liu et al., 1993, 1994), and γ-tubulin redistribution has also been noted in mouse and *Xenopus* oocytes (Palacios and Joshi, 1993; Gard, 1994).

Most of the available evidence therefore suggests that γ-tubulin caps the (–)end of a microtubule, and this is consistent with the high dependency on the microtubule end concentration observed for the binding of in vitro expressed γ-tubulin (Melki et al., 1993). Furthermore, direct visualization shows labelling of the (–)ends, with an apparent γ-tubulin:microtubule stoichiometry of approximately 17-20 γ-tubulin molecules per microtubule (H. Joshi, personal communication), in agreement with an earlier estimate using γ-tubulin complexes (see below; Stearns and Kirschner, 1994). Consequently, there is probably, within experimental error, a single γ-tubulin molecule at the (–)end of each of the thirteen protofilaments.

The in vivo and in vitro localizations imply a role for γ-tubulin in microtubule nucleation. This is supported by the effects of the disruption of the *Aspergillus* and the *Schizosaccharomyces pombe* γ-tubulin genes on nuclear division and cell viability (Oakley et al., 1990; Stearns et al., 1991; Horio et al., 1991; Horio and Oakley, 1994), and by the inhibition of microtubule nucleation by anti-γ-tubulin antibodies (Joshi et al., 1992; Julian et al., 1993; Stearns and Kirschner, 1994; Ahmed et al., 1994). γ-Tubulin is not however essential for nucleation, since α/β heterodimers can nucleate microtubules in vitro (e.g. see Algaier and Himes, 1988; Burns and Symmons, 1995), and taxol results in the spontaneous nucleation of microtubules in vivo (e.g. see De Brabender et al., 1981). The in vitro nucleation is markedly dependent on the α/β heterodimer concentration, while taxol is known to lower the steady state critical concentration (Schiff et al., 1979). The MTOC-organized γ-tubulin therefore appears to override this concentration dependency and so restrict microtubule nucleation to the organizing centers. In addition, γ-tubulin or another centrosomal protein restricts the number of protofilaments from about fourteen for microtubules assembled in vitro to the characteristic value of thirteen for in vivo microtubules (Evans et al., 1985).

While γ-tubulin appears essential for microtubule nucleation in vivo, it does not, by itself, control the actual number of assembled microtubules. For instance, the number growing off an isolated centrosome decreases with time due to the onset of dynamic instability (Mitchison and Kirschner, 1984). In addition, the relatively high cellular concentration (~0.01% of both *Xenopus* egg protein and *Aspergillus* total protein; Stearns and Kirschner, 1994; Oakley, 1994) greatly exceeds that required for a single γ-tubulin molecule at the (–)end of each assembled protofilament, while a fivefold over-expression of

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S. pombe γ-tubulin had no effect on the cell cycle time, the microtubule array, or the sensitivity of the cells to various benzimidazoles (Horio and Oakley, 1994).

It is now clear that much of the cellular γ-tubulin is dispersed in the cytoplasm and is not specifically associated with the centrosome or other MTOC. This dispersed γ-tubulin can be isolated from *Xenopus* eggs, and from *Drosophila* and human cells as a 25 S complex (termed γ-somes), which also includes various other centrosomal proteins (Raff et al., 1993; Stearns and Kirschner, 1994). This dispersed γ-tubulin can be recruited to the centrosome from *Xenopus* egg extracts by addition of KCl-stripped centrosomes, by centriole-containing sperm heads, and by *S. pombe* spindle pole bodies (Masuda et al., 1992; Ohta et al., 1993; Felix et al., 1994; Stearns and Kirschner, 1994). One consequence of this recruitment is an enhancement of the ability of γ-tubulin to promote microtubule nucleation: the unassembled γ-somes bind to assembled microtubules but fail to nucleate assembly (Stearns and Kirschner, 1994). This recruitment is faster from *Xenopus* mitotic extracts (Stearns and Kirschner, 1994), and this superficially mimics the way that the centrosome enlarges at prophase with the consequential increase in the number of nucleated microtubules (Kuriyama and Borisy, 1981). Furthermore, the dependency of the sperm-induced assembly on Mg^{2+}-ATP (Stearns and Kirschner, 1994) strongly suggests that the recruitment requires the phosphorylation of one or more of the centrosomal proteins. Indeed, the complexes induced using spindle pole bodies purified from mitotic and non-mitotic cells both contain γ-tubulin, although only those induced with the mitotic pole bodies label with a phosphoprotein-specific antibody or are able to nucleate microtubule assembly (Masuda et al., 1992). The potential importance of both γ-tubulin recruitment and the phosphorylation of a high molecular mass phosphoprotein epitope is reinforced by the blockage of centrosome-induced recruitment by protein kinase inhibitors (Ohta et al., 1993), the enhancement of the nucleating activity on addition of the purified $p34^{cdc2}$/cyclin C complex but not of MAP kinase (Ohta et al., 1993), the correspondence between microtubule nucleating activity and the reactivity to phosphoprotein-specific antibodies (Masuda et al., 1992; Stearns and Kirschner, 1994; Felix et al., 1994), and by the in vivo evidence that phosphorylation modulates the activity of the mitotic centrosome (Centonze and Borisy, 1990).

In summary, γ-tubulin is recruited to MTOCs where it seems to play a key role in microtubule nucleation, with this activity being modulated by the phosphorylation of unidentified centrosomal epitope(s). This evidence is largely derived from genetic analysis and immunological localization. By contrast, there has been almost no biochemical characterization of γ-tubulin, including whether or not γ-tubulin can be phosphorylated, due to the inherent difficulties in either purifying or expressing the necessary amounts. The probable properties of γ-tubulin, including its role in regulating microtubule assembly, can however be addressed by inspecting the conservation of the available γ-tubulin sequences, the homologies between the α-, β- and γ-sequences, and the known properties of the α- and β-tubulins.

γ-TUBULIN SEQUENCES AND FUNCTIONAL DOMAINS

Comparison of individual γ-tubulin sequences shows that they are highly conserved (e.g. see Zheng et al., 1991; Stearns et al., 1991; Fuchs et al., 1993; Luo and Perlin, 1993; Maessen et al., 1993). The availability of γ-tubulin sequences from a diverse range of organisms, which can be readily aligned with minimal insertions or deletions, now permits a more detailed analysis (Table 1). Consensus sequences have been constructed using all of the available α-, β- and γ-tubulins sequences (shown in Fig. 1) and used to inspect the individual sequences (see Table 1 for details). This analysis shows that the γ-tubulins are marginally more like the β- than the α-tubulins: reciprocal comparisons yield β/γ homologies of 0.352 and 0.368 compared with values of 0.334 and 0.354 for the α/γ comparisons (Table 1). By contrast, the α/β reciprocal comparisons (0.427 and 0.436) show that the α- and β-tubulins are more similar to each other than either is to the γ-tubulins. The α-, β- and γ-tubulins are clearly members of the same family, and it is therefore reasonable to predict that they have similar tertiary structures and related biochemical properties.

The γ-tubulin sequences contain two remarkably divergent peptides (γ-Peptide A and γ-Peptide B) which lie approximately two thirds along the primary sequence (arrows, Fig. 1), and which have a variable number of inserted or deleted residues (Table 2). Consideration of the α- and β-sequences has indicated that such heterogeneous peptides tend to lie on the exposed surface of the assembled microtubule (Burns, 1991). Indeed, the α-peptide equivalent to γ-Peptide A contains an α-chymotryptic cleavage site which is accessible in both unassembled and assembled subunits (α-Tu:Y^{339}; Mandelkow et al., 1985).

The sequence N-terminal to γ-Peptide A shows a markedly higher homology to the α- and β-sequences than those towards the C terminus (N terminus: 0.394 and 0.415 for the α/γ comparisons, and 0.407 and 0.434 for the β/γ comparisons; C terminus: 0.226 and 0.235 for the α/γ comparisons, and 0.249 and 0.245 for the β/γ-comparisons; see Table 1). Detailed inspection (not presented) based upon sub-dividing the sequences into 20-residue peptides confirms that γ-Peptide A marks the transition between the 'high' and 'low' homology regions. Indeed, only one peptide towards the C terminus shows a marked homology, and it is contiguous with a tetrapeptide (Peptide 10; Fig. 1) implicated in releasing the

Table 1. Comparison of the homologies between the α-, β- and γ-tubulin sequences

	N-terminal domain	C-terminal domain	Complete sequence
Consensus α- vs β-tubulins	0.465	0.354	0.427
Consensus β- vs α-tubulins	0.471	0.367	0.436
Consensus α- vs γ-tubulins	0.394	0.226	0.334
Consensus γ- vs α-tubulins	0.415	0.235	0.354
Consensus β- vs γ-tubulins	0.407	0.249	0.352
Consensus γ- vs β-tubulins	0.434	0.245	0.368

The fraction was calculated of the available sequences with a residue at specific position which was identical to the respective consensus sequence (see Fig. 1), and then averaged for all positions within the sequence. For example, the average probability that a specific β-tubulin residue will be identical to the consensus α-tubulin residue is 0.427. The reciprocal comparisons, i.e. all α-tubulins sequences compared with the consensus β-sequence and all β-tubulin sequences compared with the consensus α-sequence, yield very similar values (e.g. 0.427 vs 0.436). Also shown are the equivalent values for the N- and C-terminal domains, defined as those residues N-terminal and C-terminal to γ-Peptide A (see Fig. 1 and text).

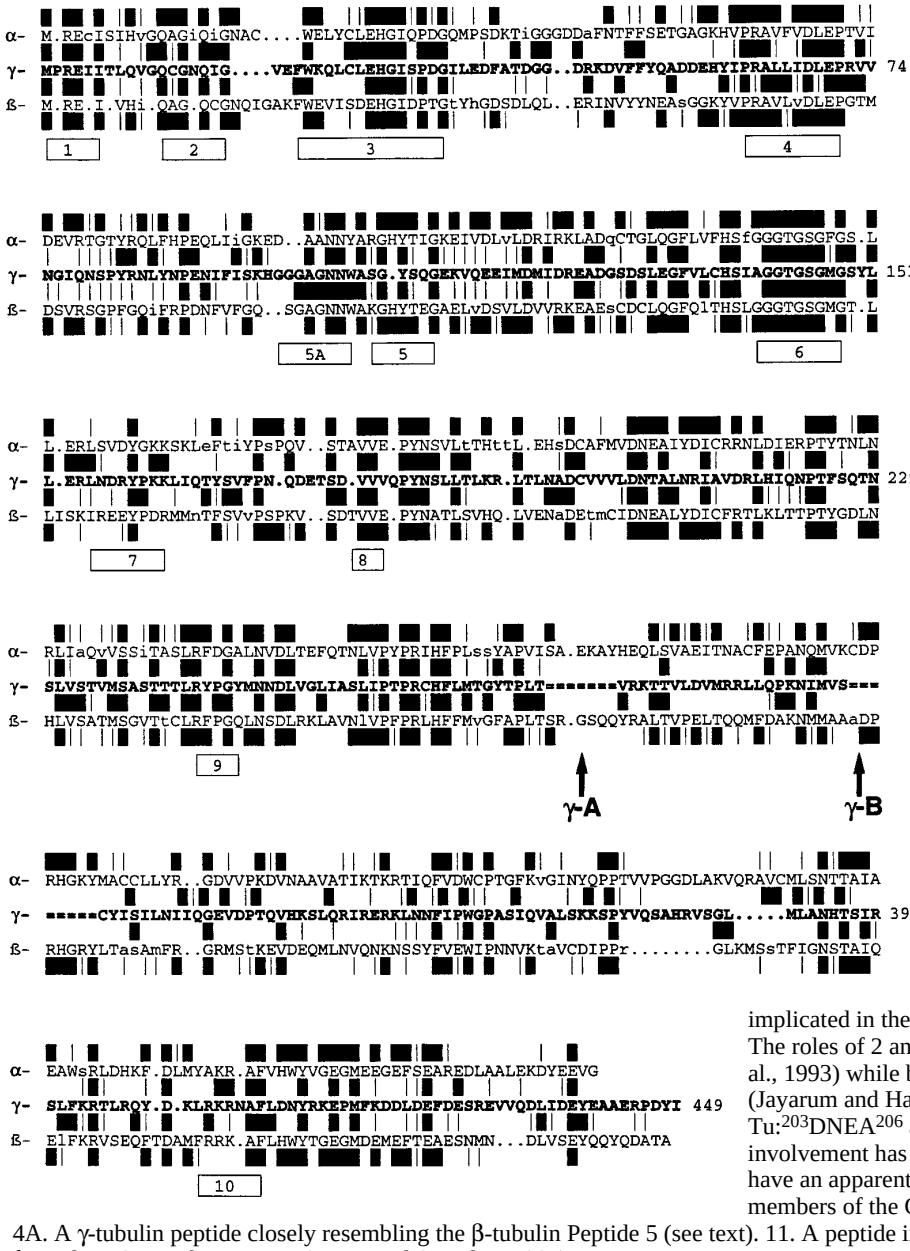

Fig. 1. Comparison of the consensus sequences of α-, β- and γ-tubulin. Consensus sequences were constructed from the 48-55 α-, 65-71 β- and 18 γ- partial or full-length tubulin sequences currently available (see Table 2 for identification of the γ-tubulin sequences), omitting the hyper-variable C-terminal peptides. The sequences were aligned manually to maximize the homologies between the three family members. Gaps (.) were inserted to maximize this alignment, and ambiguities in deriving the α- and β-consensus sequences are shown by lower-case letters. Two highly diverse γ-tubulin peptides, labelled γ-A and γ-B (and referred to in the text as γ-Peptide A and γ-Peptide B) are shown; the heterogeneity of these sequences is shown in Table 2. The upper line of symbols compares each of the available α-tubulin sequences with the consensus β-tubulin sequence. Filled blocks mark those residues in which the consensus α-sequence is identical to the consensus β-sequence, and vertical lines mark the positions in which the consensus β-tubulin residue is present in at least one of the available α-tubulin sequences. The subsequent lines of symbols use the same nomenclature but compare the available γ-tubulin sequences with the consensus α-sequence and with the consensus β-sequence, and compare the available β-tubulin sequences with the α-consensus. The numbered blocks indicate peptides with known or presumed functions: 1. A peptide implicated in the autoregulation of β-tubulin translation (Yen et al., 1988); 2-10. Peptides implicated in the binding or the hydrolysis of GTP by β-tubulin. The roles of 2 and 4-9 have been discussed elsewhere (Burns et al., 1993) while base-binding to 3 has been recently reported (Jayarum and Haley, 1994). Two additional peptides (β-Tu:^{203}DNEA^{206} and β-Tu:^{295}NKAD^{298}) are not shown, since their involvement has since been questioned (see text) even though they have an apparent homology with the motifs characteristic of members of the GTPase super-family (Sternlicht et al., 1987). 4A. A γ-tubulin peptide closely resembling the β-tubulin Peptide 5 (see text). 11. A peptide implicated in the release of α-, β- and γ-tubulin from the TCP1α chaperonine (Burns and Surridge, 1994).

nascent tubulins from the TCP1 chaperonine (Burns and Surridge, 1994). γ-Tubulin can therefore be considered as having two functional domains separated by γ-Peptide A.

The minimal homology of the γ-tubulin C-terminal sequence with the α- or β-equivalents (Table 1) indicates that it specifies the interactions with the other centrosomal proteins. One of the peptides of the C-terminal domain is particularly divergent (^{301}KNVMVSTGRDRQTNHC^{316} in human and other metazoan γ-tubulins but ^{300}KRMVSINPSKSSC^{315} or ^{303}KNQMVSVNPSKKSC^{316} in *Aspergillus* and *S. pombe* γ-tubulins). This heterogeneity suggests either that this peptide is under minimal selective pressure or that it may contribute to the respective binding of the vertebrate and fungal γ-tubulins to the centrosomal and the spindle pole body proteins. Furthermore, the divergence of the sequences of the C-terminal domains of the α- and β-tubulins from that of the γ-tubulins (Table 1) suggests that the α- and β-subunits may be unable to bind directly to the centrosomal proteins, and that this effectively restricts microtubule assembly to MTOCs containing activated γ-tubulin.

By contrast, the sequence of the γ-tubulin N-terminal domain exhibits a higher homology to the α- and β-sequences (Table 1). It is therefore likely to contribute to the binding of the initial subunit of the subtending protofilament. Proteolytic, crosslinking and immunological studies have indicated that the α- and β-subunits of protofilaments and assembled microtubules have a parallel orientation, with a C-terminal domain of one α-subunit interacting with an N-terminal domain of the adjacent β-subunit, and vice versa (Kirchner and Mandelkow, 1985; Mandelkow et al., 1985). The boundary between the N-

Table 2. Comparison of the γ-tubulin hinge peptides and the flanking residues

	γ-Peptide A	γ-Peptide B
Drosophila 1	TGYTP LTSDS DIHTQ QLVNV RKTTV	MVSTG PDKSN H--HC YISIL
Drosophila 2	TGYTP LMSDC E--AK TKTSV RKTTV	MVSAL TDKQS R--QC FVSIL
Human	TGYTP LTTD- ----Q SVASV RKTTV	MVSTG RDRQT N--HC YIAIL
Mouse	TGYTP LTTD- ----Q SVANV RKTTV	MVSTG RDRQT N--HC YIAIL
Xenopus	TGYTP LTTDN IDQAK T---V RKTTV	MVSTG RDRQT N--HC YIAIL
Chlamydomonas	TGYTP LTAEN AAGQV T-SNI RKTTV	MVSTH TKSRD AANAK YISIL
Arabidopsis 1	TGYTP LTVER QANV- ----I RKTTV	MVSSY ARNKE ASQAK YISIL
Arabidopsis 2	TGYTP LTVER QANV- ----I RKTTV	MVSSY ARNKE ASQAK YISIL
Anemia	TGYTP LTVER QANA- ----I RKTTV	MVSSY ARTKE ASQAK YISIL
Cyanidium	TGYTP LTLLE -EDRA GTSQV TKTTV	MVSCS TRKGC ----- YISIL
Euplotes	TGYTP LSLDQ KFNS- ----V RKTTV	MVTGA VKKGA ----- YMSIL
Plasmodium	TSYTP ITIDK HISN- ----V QKTTV	MVSAP VRRGM ----- YISIL
Aspergillus	TSYTP FTGDN IDQAK T---V RKTTV	MVSIN PSKSS ----C YISIL
Ustilago	TSYTP FTSDK -EQAK A---V RKTTV	LVSMP TTPPS RH-AC YISIL
S. pombe	TSYTP FTNQQ VEEAK A---I RKTTV	MVSVN PSKKS ----C FISIL
Cochliobolus	TSYTF SGENV -EQAK T---V -KTTV	MVSTN PTSHK S---C YMSIL

The locations of γ-Peptide A and γ-Peptide B are shown in Fig. 1. Sequences from the Swissprot data base, with the exceptions of mouse (Joshi, 1993), *Cyanidium* (Y. Akkari, B. R. Oakley and R. G. Burns) and *Chlamydomonas* (Dr Carolyn Silflow). The analysis shown in Table 1 was based upon the sequences of these γ-tubulins supplemented with the unpublished partial sequences of the maize and diatom γ-tubulins (Dr Zach Cande). Two additional sequences (*C. elegans* and *S. cerevisiae*) have been omitted from the current analysis as each only shares about 40% identity with the other γ-tubulins and they are only 32% identical to each other (see text).

and C-terminal domains of the α- and β-tubulins is unknown, although the γ-tubulin analysis suggests that it may correspond to the α- and β-homologues of γ-Peptide A and γ-Peptide B.

This γ-tubulin analysis has excluded two presumptive γ-tubulin sequences: *Caenorhabditis elegans* (GenBank accession number: Z22179) and *Saccharomyces cerevisiae* (U14913). Both are only 38-42% identical to the *Aspergillus* and human γ-tubulins (alignment: gcg Pileup program, default parameters) against the 68% identity of a *Aspergillus*/human comparison, and the alignments require very many more insertions or deletions. Furthermore, the *Caenorhabditis* and *S. cerevisiae* sequences are only 32% identical to each other. These two additional sequences are therefore as divergent from each other as they are from other γ-tubulins and from the α- and β-tubulins. As with the comparison of the γ-tubulins, much of the sequence identity lies within the N-terminal domain and is largely associated with peptides implicated in binding of GTP (see below). It is therefore probable that the *Caenorhabditis* and *S. cerevisiae* sequences represent two additional members of the tubulin super-family. It will be important to establish whether such sequences are present in other organisms and the functional properties of the gene products.

GTP AND γ-TUBULIN

GTP binding is a key property of the α- and β-tubulins, with the GTP bound to the α-subunit being experimentally non-exchangeable, while the GTP bound to the β-subunit is exchangeable and is hydrolyzed to GDP during microtubule assembly. The low cellular abundance of γ-tubulin and the dependency of expression on the TCP1 chaperonine (Melki et al., 1993) have hindered any direct analysis of the properties of the purified protein, including its GTP-binding properties. However, even the β-tubulin GTP-binding site has only been characterized by indirect methods (nucleotide binding assays, photoaffinity labelling, sequence analysis, and site-directed mutagenesis). In summary, four β-tubulin peptides have been implicated by photoaffinity labelling (Peptides 2, 3, 4 and 7; Fig. 1), two (Peptides 8 and 9) by homology with other NTPases, and two others (Peptides 5 and 6, see below) by site-directed mutagenesis coupled with the biochemical analysis of the purified yeast tubulin. It is still uncertain whether each of these peptides, each of which lies within the N-terminal domain, contributes directly to the GTP-binding site. For example, two additional peptides (^{203}DNEA^{206} and ^{295}NKAD^{298}) were previously implicated by analogy with the consensus peptides characterizing the GTPase superfamily proteins (Sternlicht et al., 1987); more recent work involving the expression of site-directed β-tubulin mutants in yeast followed by protein purification, GTP-binding assays and in vitro microtubule assembly have shown that neither peptide contributes directly to the GTP-binding site (Sage et al., 1995).

Many of the peptides exhibiting the highest homologies between the α-, β- and γ-tubulins, and potentially having a shared functional role, correspond with the peptides implicated in defining the β-tubulin GTP-binding site (Fig. 1). It is therefore highly probable that γ-tubulin, in common with α- and β-tubulin, binds GTP. This conclusion is indirectly supported by the observation that the amount of in vitro translated γ-tubulin which elutes with the properties of the native protein increased when the buffer contained Mg^{2+}-GTP and Mg^{2+}-ATP rather than just Mg^{2+}-ATP (Melki et al., 1993). In addition, GDP inhibits the ability of isolated centrosomes to nucleate microtubules (Nakazawa et al., 1994), although it remains uncertain whether this effect directly involves γ-tubulin.

Analysis of whether γ-tubulin hydrolyzes GTP is complicated by the observation that some of the γ-peptides more closely resemble those of α-tubulin (e.g. γ-tubulin Peptides 2 and 8), while others (e.g. γ-Peptides 4 and 9) are more akin to the β-sequences. Analysis of Peptide 5 (Fig. 1) does, however, provide an important clue about whether γ-tubulin hydrolyzes GTP, since the β-peptide (β-Tu:^{103}KGxxxxG^{109}) is postulated to coordinate the β- and γ-phosphates during the hydrolytic mechanism. Its role has been inspected by applying site-directed mutagenesis to the yeast β-tubulin gene, followed by transfection of *S. cerevisiae*, purification of the tubulin, and direct analysis of the nucleotide binding and the assembly properties (Davis et al., 1994). Three mutations (T^{107}G, T^{107}K,

and $T^{107}W$) resulted in a 3- to 7-fold higher rate of GTP hydrolysis by microtubules assembled to steady state, increased the critical concentrations for assembly, and enhanced the dynamic instability, yet the GTP binding constants to unassembled tubulin dimers were unaltered. Peptide 5 therefore appears to be important for the assembly-dependent GTP hydrolysis. The equivalent γ-tubulin peptide is markedly different and contains a deletion (^{106}SG-YSQG^{111}): γ-tubulin is unlikely to be a GTPase.

The sequence of the γ-peptide immediately N-terminal to Peptide 5 (labelled Peptide 5A; γ-Tu:^{97}GGGAGNN^{102}) is strikingly similar to that of β-tubulin Peptide 6 (β-Tu:^{139}GGGTGSG^{145}), which is presumed to form a phosphate-binding loop. Indeed, a mutation (β-Tu:$T^{143}G$) within this β-peptide reduces the GTP binding by the purified yeast tubulin, reduces the rate of GTP hydrolysis at steady state, and suppresses the dynamic instability (C. A. Doughtery, C. R. Sage, A. Davis and K. F. Farrell, personal communication). γ-Tubulin is therefore significantly different from either the α- or β-subunits in that its sequence contains two glycine-rich loops (Peptides 5A and 6), and it is therefore probable that the interaction between GTP and γ-tubulin is subtly different from that with either α- or β-tubulin. In particular, the presence of two glycine-rich loops suggests that γ-tubulin may be capable of forming two types of interaction with the phosphoryl groups.

The assembly-dependent GTP hydrolysis by the β-subunit is crucial to the dynamic behaviour of an assembled microtubule. This hydrolysis, or the subsequent release of the P_i product (Caplow et al., 1989; Melki et al., 1990), increases the rate at which assembled tubulin α/β heterodimers dissociate from the microtubule end (see Carlier, 1989; Bayley et al., 1990), and this underlies the behaviour known as dynamic instability. This increase in the subunit dissociation rate constant strongly suggests that GTP hydrolysis, or P_i release, induces the conformational change, and this change has been interpreted as causing individual protofilaments to change from a 'straight' to a 'curved' conformation (Melki et al., 1989; Diaz et al., 1993). A conformational change has also been proposed to occur before assembly in response to GTP binding to the exchangeable site on β-tubulin and the elevated temperature needed to induce assembly (Carlier, 1983; Burns and Symmons, 1995). This may represent the reverse of the straight to curved change (Shearwin and Timasheff, 1992; Burns and Symmons, 1995). One attractive possibility is that both changes involve a movement of the nucleotide-binding N-terminal domain (see Fig. 1) relative to the C-terminal domain. The remarkable conservation of the β-tubulin sequences must reflect the requirements of these conformational change(s) as well as those imposed by the interactions with GTP, α-tubulin subunits, and other proteins. The α- and γ-tubulins are as highly conserved as the β-tubulins, and this suggests that the structural constraints which have restricted the phylogenetic diversification of β-tubulin also apply to the α- and γ-subunits.

DOES γ-TUBULIN EXIST IN DIFFERING CONFORMATIONAL STATES?

The evidence that GTP is probably not hydrolyzed by γ-tubulin might imply that this subunit is unable to undergo a conformational change. The same argument would apply to the α-subunit, yet there is evidence, from an analysis of dynamic instability, that α-tubulin may exist in more than one conformational state. The various models of this microtubule behaviour (e.g. see Bayley et al., 1990) require the nucleotide status of one β-subunit to influence the status of the β-subunit of the next heterodimer. Consideration of the microtubule geometry shows that this must either occur tangentially, with one β-subunit activating a β-subunit in an adjacent protofilament, or involve the intervening α-subunit if it occurs along individual protofilaments. The β-subunit:β-subunit model would have to accommodate variations in the nature of the β:β interaction resulting from the variable number of protofilaments observed both in vivo and in vitro. Consequently, the transmission probably occurs via the intervening α-subunit, and indicates that α-tubulin is capable of undergoing a conformational change. This α-tubulin change is probably structurally related to the β-tubulin change, since the α- and β-peptides sharing the highest homology largely correspond to those implicated in the GTP-binding site (Fig. 1), and this in turn implies that the α-tubulin conformational change may alter the properties of the non-exchangeable GTP-binding site.

If α- and β-tubulin can both exist in alternative conformation states, then this may also be a property of γ-tubulin. This would be consistent with the homologies between the α-, β- and γ-peptides implicated in binding GTP (Fig. 1), while the pair of glycine-rich loops (Peptides 5A and 6) may provide alternative ways of coordinating the phosphoryl residues. Such a conformational change would, by analogy with the α- and β-subunits, involve a displacement of the N-terminal domain relative to the centrosomal-binding C-terminal domain, and so affect the binding by γ-tubulin of the terminal subunit of the subtending microtubule. Applying the protofilament straight and curved nomenclature to the potential γ-tubulin conformations suggests that microtubules can only be nucleated by γ-tubulin in the straight conformation. Consequently, as the microtubule-nucleating activity can be induced by the phosphorylation by the $p34^{cdc2}$/cyclin B complex of a high molecular mass centrosomal protein (Ohta et al., 1993), this phosphorylation probably induces γ-tubulin to undergo the curved to straight conformational change. This would be equivalent to the change in β-tubulin in response to GTP-binding (Shearwin and Timasheff, 1992; Burns and Symmons, 1995). Phosphorylation, by modulating the γ-tubulin conformation, would act as an on/off switch controlling the ability of centrosomal γ-tubulin to nucleate microtubule assembly.

γ-TUBULIN AND THE CONTROL OF DYNAMIC INSTABILITY

γ-Tubulin was initially detected as an allelic suppressor of a temperature-sensitive mutation (*benA33*) of the principle isoform of *Aspergillus* β-tubulin (Oakley and Oakley, 1989). Consideration of the properties of the benA33 phenotype not only provides further evidence for the conformational plasticity of γ-tubulin but also offers a fascinating insight into its potential role in vivo. The microtubules of the benA33 mutant were hyperstable at the elevated temperature and were resistant to benzimidazole and to other structurally unrelated antimitotic drugs (Oakley and Morris, 1981). This suggests that

the subunits had failed to undergo the hydrolysis-dependent conformational change, a possibility which is reinforced by the mapping of the *benA33* mutation to $G^{134}K$ (Oakley, 1994), a residue which lies immediately N-terminal to the glycine-rich peptide implicated in phosphate binding (Peptide 6, Fig. 1).

The suppression of the phenotypes of *benA33* and other *Aspergillus* β-tubulin mutants by the α-tubulin mutant (*tubA*1) has been interpreted as indicating the *tubA*1 mutation destabilizes the microtubules, so compensating for the hyperstabilization resulting from the *benA33* mutation (Oakley and Morris, 1981). This mechanism cannot apply to the temperature-sensitive product of the mutant γ-gene (*mipA*), since γ-tubulin is specifically localized to the spindle pole body and therefore cannot induce destabilization by directly interacting with each of the assembled *benA33* β-tubulin subunits. Furthermore, *mipA* not only suppresses the benA33 phenotype but it also confers cold-sensitivity to, and reduces the benzimidazole-resistance of, a variety of other β-tubulin mutations (Weil et al., 1986; Oakley and Oakley, 1989).

This ability of γ-tubulin to influence the properties of the subtending microtubule clearly indicates that the changed interaction between the γ-tubulin N-terminal domain and the subtending α/β heterodimer is transmitted to each of the other α/β heterodimers of the assembled microtubule. Specifically, it is proposed that the *mipA* mutation affects the interaction with the subtending α/*benA33*:β heterodimer and that this permits it to undergo the normal conformational change in response to the GTP hydrolysis. This perturbation must then be transmitted to the other α/*benA33*:β heterodimers of the subtending microtubule to result in the assembly of an intrinsically unstable microtubule. This novel mechanism, based on the phenotypic effects of the *mipA* gene and representing a simple extension of the transmission implicit in models of dynamic instability, indicates that γ-tubulin may play a key role in regulating the intrinsic stability of assembled microtubules.

The potential of the microtubule to exhibit dynamic instability may therefore depend on the properties of the γ-tubulin subunits at the microtubule (−)ends. Indeed, some microtubules originating from the MTOC are unusually stable (e.g. see Schultze and Kirschner, 1987) and become the substrates for tyrosylation and acetylation (e.g. see Gundersen et al., 1984; Webster and Borisy, 1989). The evidence from the *mipA* mutation offers a mechanism by which this control may be effected by γ-tubulin although other mechanisms, including the microtubule associated proteins, may contribute to the stabilization.

It has already been noted that the curved to straight transition of γ-tubulin, potentially controlled by the phosphorylation of a centrosomal protein, represents a simple on/off switch. This conformational change does not readily account for the potential control of the intrinsic stability of the subtending microtubule. The straight conformation may therefore exist in two sub-states, one of which permits the assembly of microtubules exhibiting dynamic instability, and a second which results in the assembly of stable microtubules. The control of these two sub-states is unknown, but it may be significant that all γ-tubulins contain the peptide ^{270}(L/M/V/I/T)T(S/G)Y^{273}, which lies immediately N-terminal to γ-Peptide A. This sequence resembles the (L/M)T(G/E)Y motif of the MAP kinases (12 sequences, Swissprot data base), which are activated by the dual phosphorylation of the conserved threonine and tyrosine residues (see Taylor and Radzio-Andzelm, 1994). Significantly, this ^{270}xTxY^{273} motif is conserved in all γ-tubulins but is absent from all α- and β-tubulins. One interesting possibility is that a component of the MAP kinase cascade phosphorylates γ-Tu:T^{271} and/or γ-Tu:Y^{273} and so switches off the ability of γ-tubulin to nucleate dynamically unstable microtubules. This mechanism would be distinct from, and additional to, the proposed regulation by phosphorylation of dynamic instability by the modulation of the (+)end catastrophy and rescue frequencies (Cassimeris, 1993) in which the phosphorylation is proposed to enhance the dynamic instability. Both mechanisms may contribute to the proposed modulation of microtubule stability by extracellular signals (Kirschner and Mitchison, 1986). The proposed modulation of the γ-tubulin properties may contribute to the enhancement of the fraction of stable, detyrosinylated microtubules following cell damage (Gundersen and Bulinski, 1988) and by components of calf serum, including TGF-β1 and TGF-β2 (Gundersen et al., 1994).

In summary, analysis of the α-, β- and γ-tubulin sequences indicates that the C-terminal domain of γ-tubulin interacts with the centrosomal (or spindle pole body) proteins, while the N-terminal domain binds to the α/β heterodimer of the subtending microtubule. The sequence analysis also suggests that γ-tubulin binds GTP but does not, in common with the α-subunit, hydrolyze it. Consideration of the properties of microtubule assembly indicates that γ-tubulin can exist in two conformational states, only one of which effects microtubule nucleation, and that the transition between the two states is controlled by the phosphorylation of a centrosomal protein. Finally, the phenotypic effects of a γ-tubulin mutant, coupled with the mechanism underlying dynamic instability, suggests that there are two sub-states of the conformational state which supports microtubule assembly. One of these sub-states is proposed to nucleate the assembly of dynamically unstable microtubules while the other results in the assembly of intrinsically stable microtubules. These two sub-states may be regulated by the phosphorylation of γ-tubulin by a component of the MAP kinase cascade. The similarities of the α-, β- and γ-tubulin sequences confirm that these three proteins are members of a common family while the differences highlight the fact that they have subtly different cellular functions.

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