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Computational prediction of the three-dimensional structures for the Caenorhabditis elegans tubulin family

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In this article we characterize, from a structural point of view, all 16 members of the tubulin gene family of Caenorhabditis elegans *(9 α-tubulins, 6 β-tubulins, and 1 γ-tubulin). We obtained their tertiary structures by computationally modifying the X-ray crystal structure of the pig brain α/β-tubulin dimer published by Nogales et al. [*Nature (London) *1998;***391***:199–203]. Our computational protocol involves changing the amino acids (with MIDAS; Jarvis et al.,* UCSF MIDAS. *University of California, San Francisco, 1986) in the 3D structure of pig brain α/β-tubulin dimer followed by geometry optimization with the AMBER force field (Perlman et al.,* AMBER 4. *University of California, San Francisco, 1990). We subsequently analyze and compare the resulting structures in terms of the differences in their secondary and tertiary structures. In addition, we compare the pattern of hydrogen bonds and hydrophobic contacts in the guanosine triphosphate (GTP)-binding site for all members of the tubulin family. Our computational results show that, except for γ-tubulin, all members of the* C. elegans *tubulin family have similar secondary and 3D structures and that the change in the pattern of hydrogen bonds in the GTP-binding site may be used to assess the relative stability of different α/β-tubulin dimers formed by monomers of the tubulin family. © 2000 by Elsevier Science Inc.*

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INTRODUCTION

Microtubules are essential elements in the life cycle of an eukaryotic cell.[1] The microtubules form an extensive network through the cytoplasm out to the cell edges. A microtubule consists of a hollow cylinder made of 13 protofilaments that are built up of α/β-tubulin dimers.[2,3] Microtubules are dynamic molecular aggregates controlled by accessory proteins (MAPs, which assemble/disassemble them in response to cellular conditions); they perform highly critical cellular functions such as in cellular movement, axonal outgrowth, cell division, and intracellular transport, and they provide basic cytoarchitecture.[1]

The nematode *Caenorhabditis elegans* has several types of microtubules and MAPs, such as kinesins, which have been characterized both *in vitro* and *in vivo*.[4–9] Like in other higher organisms, *C. elegans* tubulins are encoded by a family of genes. Our group has characterized five *C. elegans* α-tubulin-encoding genes: *tba-1*,[10] *tba-2*,[11] *tba-3*,[12] *tba-4* and *tba-6*.[13] Herein we discuss the structure of their coding region and the regulatory sequences in the 5′ upstream promoter region of the α-, β-, and γ-tubulin isotypes. The analysis of the amino acid sequences of α- and β-tubulin isotypes shows that their primary structure is highly conserved in the (amino) N-terminal and intermediate sequence, but is highly divergent in the (carboxyl) C-terminal sequences (see below). It was suggested that γ-tubulin caps the (−) end of a microtubule.[14] In order to understand the differences between the primary structures of different isotypes we constructed their 3D structures by modifying the X-ray crystal structure of the pig brain α/β-tubulin dimer[15] (Brookhaven DataBank: 1TUB) by computation as follows: we substituted, added, or deleted residues in the pig brain 3D structure according to the primary structure of the other α- and β-tubulin isotypes. Subsequently, we performed a geometry optimization with the AMBER united-atom force

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field[16] to remove the van der Waals overlaps introduced by the substitution, addition, or deletion of amino acids.

The article is organized as follows: in the second section we introduce all members of the *C. elegans* tubulin gene family and discuss the intron/exon boundary in *C. elegans* genomic sequences, and the homology of the primary structures of the *C. elegans* tubulin family with those found in other organisms. In the third section we describe the computational methodology used to generate the 3D structure for *C. elegans* tubulin isotypes and characterize each isotype in terms of its secondary structure (the size of the secondary structure units and their relative 3D orientation). In addition, we discuss differences in the hydrogen-bond (H-bond) network and hydrophobic contacts for GTP (α-, β-, and γ-isotypes), guanosine diphosphate (GDP, β-isotypes), and taxol (TXL, β-isotypes) binding sites.[15] Finally, our concluding remarks are given in the final section.

CAENORHABDITIS ELEGANS TUBULIN FAMILY

α-, β-, and γ-Tubulin isotypes

The *Caenorhabditis elegans* tubulin family consists of nine α-tubulins, six β-tubulins, and one γ-tubulin.* We characterized previously five α-isotypes (*tba-1* to *tba-4*[10–12,18], *tba-6*[13]) and one γ-tubulin (*tbg-1*[19]), and we report here four new members of the *C. elegans* α-tubulin family: *tba-5*, *tba-7*, *tba-8*, and *tba-9* (see below). The β-tubulin family has six members: *tbb-1* (*mec-7*), *tbb-2*, *tbb-3*, *tbb-4*, *tbb-5* (*ben-1*), and *tbb-6*. All isotypes (except *tbb-6*) have a high homology in the primary structure. *tbb-6* has features from both α-tubulin (34% homologous with TBA1) and β-tubulin (54% with TBB1) families. The *C. elegans* tubulin family also includes one γ-tubulin gene: *tbg-1*.

Table 1. Genes of the *Caenorhabditis elegans* α- and β-tubulin famlies

Gene	Linkage group	Cosmid	cDNA	Protein size (amino acids)
tba-1	I	F26E4.8	SQ Tba77, yk210d2 yk245g5, yk289d3	449
tba-2	I	C47B2.3	yk67a8	448
tba-3	III	F56F12	SQ CBG56, yk234e1 yk341a11, yk389f10 yk50363	450
tba-4	II	F44F4.11 Y19D2B	SQ pTF4	447
tba-5	I	F16D3.1	—	447
tba-6	I	F32H2.9	SQ CBG32, yk450g5	460
tba-7	III	T28D6.2	—	444
tba-8	X	ZK899.4	—	448
tba-9	X	F40F4.5	—	460
tbb-1	X	ZK154.3	yk143g8, yk372d11 yk450h7	441
tbb-2	X	B0272.1	yk230e11, yk313f12 yk80b7	444
tbb-3	III	C36E8.5	yk103a1, yk104b4	450
tbb-4	III	K01G5.7	yk108b2, yk108d12	449
tbb-5	III	C54C6.2	yk119d7	452
tbb-6	V	T04H1.9	—	420
tbg-1	III	F48A4.8	yk496c1, yk80h7	444

Chromosome mapping and intron/exon boundaries

The *tba-1* gene, encoded by the cosmid clone F26E4, is located on chromosome I (Table 1), which also harbor *tba-2* (encoded by C47B2), *tba-5* (F16D3), and *tba-6* (F32H2). The *tba-4* gene, partially encoded by cosmid F44F4.11,† is accommodated on chromosome II. *tba-3* (also known as *mec-12*, encoded by F56F12) and *tba-7* (T28D6) are located on chromosome III. *tba-8* (cosmid ZK899) and *tba-9* (F40F4) are located on chromosome X. Table 1 shows that the genes encoding α-tubulins are distributed on four of the six linkage groups of *C. elegans* chromosomes, i.e., I, II, III, and X, and no α-tubulin gene was found on chromosomes IV and V. The α-tubulin genes *tba-1*, *tba-2*, and *tba-4* have small introns evenly spaced and distributed in different locations. However, the remaining six genes (*tba-3*, *tba-5*, *tba-6*, *tba-7*, *tba-8*, and *tba-9*) have multiple introns: 3 in *tba-3* and *tba-7*, 5 in *tba-5*, 6 in *tba-9*, 8 in *tba-6*, and 11 in *tba-8*. While the introns of *tba-8* have lengths of 50 to 100 bp, *tba-3* has four unusually large introns (1, 2.3, 0.6, and 1.6 kb). We found it interesting that the fourth intron of *tba-6* encodes in an opposite orientation a novel gene that appears to be a transcription factor.[13]

β-Tubulin isotypes are located on three of the six *C. elegans* chromosomes (Table 1). *tbb-3* (encoded by C36E8), *tbb-4* (K01G5), and *tbb-5* (*ben-1*, C54C6) are mapped on chromosome III, *tbb-6* (T04H1) on chromosome V, and *tbb-1* (ZK154) and *tbb-2* (B0272) on chromosome X. The intron/exon boundaries of β-tubulin genes are similar to those found in α-tubulin isotypes. *tbb-4* (three exons) and *tbb-3* (four exons) have very small introns (45 to 77 bp) and medium-size exons (except for the second exon in *tbb-4*, which is 806 bp). The *tbb-1*, *tbb-2*, *tbb-5* and *tbb-6* genes have a larger number of medium-size exons and introns, except for the first intron of the *tbb-5* intron, which is 1015 bp long.

Finally, the *tbg-1* gene, encoded by F58A4, is located on chromosome III (Table 1). *tbg-1* has six small exons, ranging from 104 to 312 bp, and five introns: two small (48 and 54 bp) and three of medium size (199, 287, and 555 bp).[19]

Sequence alignment and posttranslation modification sites

Color Plate 1 shows the homology of α-tubulin primary structures obtained with the CLUSTAL W program.[20] The program outputs the protein primary sequences according to their degree of similarity. An α-tubulin protein generally has 450 residues,

*Chervitz et al. suggested that there are 17 members.[17] The confusion arises from the fact that the *tba-4* gene is encoded partially in cosmid F44F4.11 (N terminal) and YACY19D2B (C terminal).

†The downstream sequence is deduced from YAC clone Y19D2B.

Table 2. Homology of the *Caenorhabditis elegans* α-tubulin primary structures of TBA1 to TBA9 with other tubulin isotypes from mouse (TBA6_mouse), *Torpedo marmorata* (TBA_torma), *Haemonchos contortus* (TBA_haeco), *Xenopus laevis* (TBA_xenla), pig brain (TBA_pig), *Paracentrotus lividus* (TBA_parli), *Drosophila melanogaster* (TBA_drom), and human (TBA1_human)[a]

Protein	TBA1	TBA2	TBA3	TBA4	TBA5	TBA6	TBA7	TBA8	TBA9
TBA1	100	97	87	96	78	75	85	77	71
TBA6_mouse	87	87	91	85	83	78	81	77	75
TBA_torma	88	87	92	86	83	77	80	77	75
TBA_haeco	86	87	99	86	81	75	81	77	74
TBA_xenla	86	86	91	85	82	78	80	77	74
TBA_pig	87	88	93	86	85	77	80	77	74
TBA_parli	85	86	90	84	82	77	79	77	73
TBA_drom	86	87	91	85	82	78	80	77	74
TBA1_human	87	86	92	84	82	77	79	76	74

[a] Entry refers to identities (%).

except for TBA6 and TBA9 (460 residues). TBA6 has a long and unusual tail, while TBA9 has a different pattern of amino acids in its primary sequence compared with other members of the family. TBA9 starts with a 20-amino acid sequence (Met1–Glu20), which is missing in other isotypes, and has an insertion of 15 amino acids (Pro94–Ser108), and two deletions of 7 (Glu320–Met326) and 23 (Thr327–Ala349) amino acids. Table 2 gives the homology of TBA1 with other members of the *C. elegans* α-tubulin family (first row), and the homology of all *C. elegans* members with α-tubulin isotypes from mouse (TBA6−mouse[21]), *Torpedo marmorata* (TBA−torma[22]), *Haemonchos contortus* (TBA−haeco[23]), *Xenopus laevis* (TBA−xenla[24]), pig brain (TAB−pig[15]), *Paracentrotus lividus* (TBA−parli[25]), *Drosophila melanogaster* (TBA−drom[26]), and human (TBA1−human[27]). Table 2 shows that TBA3 has the highest (~92%) and TBA9 the lowest (~73%) homology (percent identity) among the members of the *C. elegans* family with α-tubulins of other species.

Color Plate 2 gives the homology in the primary structures of β-tubulins. Homologies (Table 3) between *C. elegans* β-tubulin isotypes and TBB−chick,[28] TBB1−rat,[29] TBB−pig,[30] and TBB2−human[31] show that TBB2 has the highest (~92%) and TBB6 the lowest (~54%) homology with the corresponding proteins from the above-mentioned species. TBB3, TBB4, and TBB5 have the same size as α-tubulin proteins, while TBB1, TBB2, and TBB6 are smaller. TBB6 is unusually small (only 420 residues) and has four deletions of 5 (Gly223–His227), 2 (Ala254–Val255), 1 (Ala302), and 3 (Gly408–Gly410) amino acids. On the other hand, TBB5 is longer with 8 amino acids (Thr388–Thr395) than the other *C. elegans* β-tubulins.

Table 3. Homology of the *Caenorhabditis elegans* β-tubulin primary structure of TBB1 to TBB6 with other tubulin isotypes from chicken (TBB_chick), rat (TBB1_rat), pig brain (TBB_pig), and human (TBB2_human)[a]

Protein	TBB1	TBB2	TBB3	TBB4	TBB5	TBB6
TBB1	100	91	87	87	87	54
TBB_chick	92	93	88	88	88	54
TBB1_rat	92	93	87	87	88	53
TBB_pig	92	94	88	89	89	55
TBB2_human	92	94	90	90	90	54

[a] Entries refer to identities (%).

Caenorhabditis elegans γ-tubulin (TBG1) has a low homology with both α-tubulin (27% with TBA1) and β-tubulin (31% with TBB1), but also with other γ-tubulins from other species. For example, while TBG2−drome[32] is 76% homologous with TBG−xenla[33] and TBG−human,[34] *C. elegans* TBG1 is only 44% homologous with TBG2−drome, 41% with TBG−human, and 30% with TBG−yeast.[35] However, the low homology of *C. elegans* γ-tubulin with other organisms led Melki et al.[14] to suggest that the *tbg-1* gene (named δ-tubulin by Burns[36]) together with *Saccharomyces cerevisiae* γ-tubulin (also named ϵ-tubulin[36]) belong to a different class of tubulin family.

Key features of α-tubulins, such as the GTP-binding site, are conserved in all *C. elegans* isotypes (see the third section). Similarly, the cell attachment the RDG tripeptide sequence[37,38] was found in all *C. elegans* α-isotypes that also have the casein kinase[39] and tyrosine[40−42] kinase phosphorylation sites. TBA5 lacks the protein kinase C phosphorylation site.[43,44] Earlier we pointed out that the C-terminal amino acid sequences of TBA1[10] (EGNEEEGEEY) and TBA2[11] (NEGGEEEGEEY) are similar to the C-terminal amino acid sequence (EGEGEEEGEEY) of the other α-tubulin isotypes, which are expressed in the brain tissue of human, mouse, and rat. Among the new α-tubulins reported here, only TBA3 has a C-terminal amino acid sequence similar to that of the α-tubulins described above.[11]

Like α-tubulins, the β-tubulins also conserve the GTP-binding site. Fifteen N-myristoylation sites[45,46] are found in β-tubulins as compared with only 9 in α-tubulins. In addition, casein kinase II, protein kinase C, and tyrosine kinase phosphorylation sites were also identified in β-tubulins. However, except for TBB6, all other β-tubulins, as well as TBG1, lack

RGD cell attachment[37,38] and amidation[47,48] sites. Typical of all β-tubulins is the presence of the cAMP and cGMP phosphorylation site (KRIS[49–51]), also found in TBG1.

We determined next the occurrence of regulatory sequences in the promoter region of the *C. elegans* tubulin family genes. The eukaryotic transcription signal (TATA box: TATAA) is found in all α-tubulin genes. The *tba-8* promoter has three TATA boxes more than 1 kb away from the start codon (ATG), at positions −1279, −1302, and −1311. *tba-1*, *tba-5*, and *tba-7* have only one TATA box, whereas *tba-2*, *tba-4*, and *tba-6* have four, two, and three, respectively, suggesting different transcription mechanisms for these isotypes. In the promoter region of β- and γ-tubulin genes the position of the TATA boxes is far upstream from the start codon, i.e., −797 and −1033 for *tbb-2* and −1060 for *tbg-1*. The eukaryotic cell enhancer element (CAAT) sequence is distributed across different locations in the α-tubulin genes and occurs with different frequencies. For example, the *tba-5* promoter has seven enhancer sequences, *tba-2* and *tba-7* have two, *tba-3* and *tba-8* have three, and *tba-4* and *tba-6* have four and five CAAT sequences, respectively. The differences both in number and position of CAAT sequences in the promoter region suggest complex regulatory control in the expression of different tubulin isotypes.

β-Tubulin genes have at least seven CAAT enhancer elements in contrast to α-tubulin genes, which have no more than six (except for *tba-5*). *tbb-2* has 8, and *tbb-3* and *tbb-6* have 10. *tbg-1* has eight CAAT enhancer elements and only one TATA box (−1060). In addition to the *cis*-splicing mRNA found in vertebrates,[1] *C. elegans* also has *trans*-splicing machinery for mRNAs.[52] We have previously shown that *tba-1* and *tba-2* undergoes a *trans*-splicing reaction,[10–12,18] but here we note that all *C. elegans* tubulin genes have the *trans*-splicing reaction acceptor site (TTTCAGAA), a common feature of both α- and β-tubulin genes.

PREDICTION OF THE THREE-DIMENSIONAL STRUCTURE FOR *CAENORHABDITIS ELEGANS* TUBULIN FAMILY

Computational protocol

Although we have applied our computational strategy only to the tubulin family, the method is general and can be used with any protein family pending the existence of the X-ray crystal structure for at least one member of the family. This approach for studying the 3D structure of proteins originated in the idea that proteins from the same family share a high homology and presumably a large part of the secondary and tertiary structure. Thus, by performing local changes (mutations) in the structure of a known protein followed by structure relaxation (minimization), another member of the family can be obtained. The chance that the new structure is close to the real structure increases with increasing homology between the primary structures of the two proteins. We tried to minimize the uncertainty introduced by various mutations in the structure of a newly derived member by performing one mutation (residue exchange, addition, or deletion) at a time, followed by structure relaxation (i.e., energy minimization). This is particularly important when sequences of a few amino acids must be deleted or inserted. Following one mutation, we checked whether the newly added/changed amino acid accommodates the local environment (i.e., it is not interfering with neighboring residues).

A breakthrough in tubulin research was the determination of the X-ray crystal structure (at 3.7-Å resolution) of pig brain α/β-tubulin dimer (TBAB−pig) by Nogales et al.[15] However, the rather low resolution of the crystallographic data causes uncertainty in the location of some of the atoms in this protein.[15] Hence, we first added the hydrogen atoms and proceeded with the optimization[‡] (AMBER united-atom force field[16]) of the TBAB−pig dimer 3D structure (obtained from the authors), which is available now from Brookhaven Protein DataBank (1TUB). Because we used the optimized structure of the TBAB dimer as the reference structure for obtaining all other isotypes we first looked into the differences between the crystal and the optimized structures. Thus, we performed two statistical analyses: first we determined the differences between the internal coordinates of the crystal/optimized structures, and then we performed a per-residue root mean square (RMS) analysis (see below). The statistical analysis of the differences in the internal coordinates was performed with a program that we developed specifically for this purpose. The program transforms the Cartesian coordinates (PDB file) into internal coordinates and calculates separate RMS values for bond lengths, bond angles, and dihedral angles. Our analysis shows that there were unnoticeable differences in the bond lengths and bond angles, and little difference in some of the dihedral angles of the TBAB crystal and optimized structures (see below). The per-residue RMS analysis was performed with the ProFit program,[53] which uses the McLachlan fitting algorithm[54] (essentially a steepest descents minimization). The results are discussed in the following section. The X-ray crystal structure of the α/β-tubulin dimer also contains GTP (located between the α- and β-monomer units), and guanosine diphosphate (GDP) and taxol (TXL, both attached to the β-tubulin unit). TBB1 (*mec-7*) was used as the β-tubulin monomer for dimers containing TBA1, TBA2, TBA3, TBA4, TBA5, TBA7, and TBA8, while the bigger TBB3 (450 residues) is used for dimers containing TBA6 and TBA9. Our computational protocol is as follows: we used MIDAS[55] to alter the 3D structure of TBA−pig/TBB−pig dimer by replacing, deleting, or adding residues according to differences in the primary structures of TBA−pig/TBB−pig dimer and the target TBA/TBB tubulin dimer and, subsequently, we optimized the new dimer with the AMBER[16] force field to remove the van der Waals overlaps induced by residue changes. As already mentioned, in the case of deleting/adding a sequence of amino acids we deleted/added one amino acid at a time and then allowed the structure to relax by optimization after each change in the structure.

The resulting 3D structures were analyzed in terms of similarities in their secondary and tertiary structures by introducing a similarity/dissimilarity index. This index was defined in the following way: we mapped out the GTP-, GDP-, and TXL-binding sites by making an inventory of the H bonds and

[‡]A distance-dependent dielectric constant ($4r_{ij}$) was used to mimic the water environment as a continuum dielectric medium.[16] The optimization was carried on until the root mean square of the gradient dropped to less than 0.05. The CPU time for optimization increases with the number of mutated residues. For example, the optimization of the TBG1/TBB1 dimer required 17 hr of CPU time (about 90,000 iterations). Typically, between 5000 and 10,000 iterations were needed for members with high homology to TBAB−pig.

hydrophobic contacts of these ligands with the nearby residues (less than 5 Å away), and calculated the index (the sum of the squared differences) between the two maps.

TBA1, TBA2, and TBA3 were already analyzed in a previous article,[52] and thus we discuss here the other members of the α-tubulin family (TBA4 to TBA9), all members of the β-tubulin family (TBB1 to TBB6), and TBG1 γ-tubulin.

Caenorhabditis elegans α-tubulin family

TBA−pig. For completeness, we give a short description of TBA−pig, which was used as a reference for discussing structural differences in the other members of the α-tubulin family. The structure of TBA−pig contains 2 β-sheets of 6 and 4 strands flanked by 12 α-helices, and can be divided into three domains (Color Plate 3).[15] The N-terminal domain (Met1–Asp205) forms a Rossmann fold (typical for nucleotide-binding proteins) in which parallel β-strands alternate with α-helices. Helices H1 and H2 are on one side of the sheet, whereas H3, H4, and H5 are on the other. The intermediate domain (Asn206–Ala381) contains a mixture of four β-sheets and five α-helices. The domain starts with H6 and H7 and connects to H8 through a long loop positioned between the two monomers. B7 is a long β-strand that interacts with the β-sheet of the N-terminal domain. The loop between B9 and B10 includes an eight-residue insertion in the α-tubulin monomer that occludes the site where taxol binds in the β-tubulin monomer. The C-terminal domain is formed by helices H11 and H12, which are sitting on the surface of the protein and are probably involved in binding MAPs and motor proteins.[15] The loop connecting H11 and H12 is important for the interaction with the next monomer along the protofilament.

To validate the use of the optimized structure of TBA−pig/TBB−pig dimer as a reference in analyzing the structures of the *C. elegans* TBA/TBB family, we performed a statistical analysis as described in the previous section. Some differences between the two structures are to be expected because of the low resolution and the van der Waals contacts introduced by adding the hydrogen atoms. The molecular mechanics (AMBER) energy of the TBAB dimer (crystal structure) is large positive and optimization was necessary to obtain a starting structure for deriving the other members of the TBAB family. The results of the statistical analysis are given in Tables 4 and 5. The RMS between the crystal and optimized structures of TBAB is 0.7 Å (ProFit). Table 4 shows per-residue RMS values for the crystal/optimized structures of TBAB. More than half of the TBA residues (256) and 199 TBB residues have an RMS less than 0.5 Å, while only 7 TBA residues and 3 TBB residues have RMS values larger than 2 Å. The RMS for GTP is less then 0.5 Å, and that for GDP and taxol less than 1 Å. Table 5 gives the differences between the dihedral angles of the optimized/crystal structures of TBAB. For TBA 91% of the dihedral angle differences are smaller than 10°. The pattern is similar for TBB (87%), taxol and GTP (96%), and GDP (70%). Only one dihedral angle is larger than 90° for TBA, and two for TBB. We found a significant change in conformation between the crystal and optimized structures only for a few residues. For example, Thr340 (TBA) has a per-residue RMS of 2.4 Å. The N—C_α—C_β—O_γ dihedral angle is 27° in the crystal structure and −31° in the optimized structure. Thus, we assigned a difference between the two angles of 302° (because of the change in angle sign). This is the largest difference for a dihedral angle in TBA, but Thr340 is far from GTP (25 Å), GDP (58 Å), and taxol (48 Å) and consequently plays little role in the analysis of the binding sites for the ligands. Another example is Lys392 (TBB), with a per-residue RMS value of 3 Å. The differences in the dihedral angles N_ζ—C_ϵ—C_δ—C_γ and C_δ—C_γ—C_β—C_α between crystal/optimized structures, are 26 and 30°, respectively. Lys392 is also far from the three ligands: GTP (51 Å), GDP (21 Å), and taxol (38 Å). Lys430 (TBA) has a per-residue RMS of 3.4 Å and one large difference (119°) in the dihedral angle C_δ—C_γ—C_β—C_α, which is 192° in the crystal structure and −48° in the optimized structure. Lys430 is 25 Å from GTP, 51 Å from GDP, and 46 Å from taxol. According to the results of the statistical analysis we conclude that there are no significant differences between the crystal and the optimized structures of the TBA−pig/TBB−pig dimer and consequently we used the optimized structure to derive the other isotypes of the *C. elegans* TBA/TBB tubulin family.

Table 4. Per-residue RMS values between the AMBER-optimized and crystal structures of TBA_pig/TBB_pig dimer

	RMS[a]					
Molecule	0.0–0.5	0.5–1.0	1.0–1.5	1.5–2.0	>2.0	Total
TBA_pig	256	136	31	10	7	440
TBB_pig	199	183	37	5	3	427

[a] Entries represent angstroms.

TBA4. The homologies (percent identity), and the changes in the amino acid primary structure of all the α- and β-tubulins with respect to the pig brain α- and β-isotypes can be seen in Tables 2 and 3. The secondary structure of TBA4 (determined with WebLab Viewer[56]) is given in Color Plate 1 and the statistics of the changes in the amino acid character (hydrophobic/hydrophilic) are given in Table 6. One of the deleted residues (Pro222) is close to the GTP-binding site in TBA−pig (Color Plate 4), i.e., on the loop connecting H6 with H7 and only 2 residues away from Tyr224, which makes an H bond (2.0 Å) with a hydroxyl oxygen of the GTP sugar unit. Asp46 and 47 are in the loop connecting H1 with B2, a newly formed β-strand (B1′: Tyr61–Val62), and Gly444 is in the TBA4 tail. The conformation of TBA4 (i.e., the relative orientation of secondary structure elements) does not change much as compared with the "parent" TBA−pig. There is some conformational change in the 42-residue loop connecting H1 with B2, but we expect that the stability of the TBA4/TBB1 dimer is close to that of TBA−pig/TBB−pig because the GTP-binding site is not significantly changed (Table 7, and see below).[§]

TBA5. There is no noticeable difference between the 3D structures of TBA5 and TBA−pig. As in TBA4, our analysis shows little modification of the GTP-binding site in TBA5 (Table 7, and see below). The mutations carried out in the vicinity of the GTP-binding site are between amino acids with the same hydrophilic/hydrophobic character (Table 6). The

[§]Further computational details as, e.g., mutated structures and the full map of GTP/GDP/TXL binding sites for all tubulin isotypes are available on request from the authors.

Table 5. Difference in dihedral angles between the AMBER-optimized and crystal structures of TBA_pig/TBB_pig dimer

	Difference in dihedral angles[a]							
Molecule	0.01–0.17	0.18–0.52	0.53–1.04	1.05–1.57	1.58–2.09	4.72–5.23	5.24–5.75	Total
TBA_pig	3131	279	22	3	1	1	1	3438
TBB_pig	2918	379	41	11	2	1	4	3356
GTP	26	1	—	—	—	—	—	27
GDP	16	5	2	—	—	—	—	23
TXL	51	2	—	—	—	—	—	53

[a] Entries are in radians.

Table 6. Statistics of the change in the hydrophilic/hydrophobic character of the mutated amino acids in *C. elegans* tubulin family with respect to TBA_pig/TBB_pig dimer

	Changed amino acids					Difference in the protein length[3] (amino acid)
Protein	Total	phy[1] → pho[2]	pho → phy	phy → phy	pho → pho	
TBA4	61	4	14	27	16	−4
TBA5	76	6	15	22	33	−4
TBA6	94	11	15	34	34	+9
TBA7	83	12	21	22	28	−7
TBA8	102	17	20	38	27	−3
TBA9	64	9	6	18	31	+9
TBB1	33	6	6	10	11	−4
TBB2	31	8	8	7	8	−1
TBB3	44	9	5	19	11	+5
TBB4	46	8	10	18	10	−4
TBB5	41	10	3	19	9	+7
TBB6	179	23	39	67	50	–25
TBG1	263	50	53	88	72	−10

[1] hydrophilic character.
[2] hydrophobic character.
[3] with respect to TBA_pig/TBB_pig.

only significant difference with respect to TBA−pig is the extension of H12 by 4 amino acids (Color Plate 1).

TBA6. TBA6 has 2 amino acids (Trp and Ile, Table 6) inserted between Met1 and Arg2, and 7 amino acids in the TBA−pig tail between Glu447 and Gly448. The 43-residue-long segment connecting H1 and B2 contains 16 mutations, confirming the suggestion by Burns and Surride[57] that this loop (inside of the microtubule surface) can easily accommodate amino acid mutation, insertion, and deletion. In TBA6 the conformation of this loop changes slightly (Color Plate 5) as compared with TBA−pig (Color Plate 3). The loop in TBA6 has four extra glutamates (Glu38, 44, 45, and 47), a histidine (His36), and an exchange of charged residues (Lys40 ⇒ Asp). Hence, there are reasons to believe that this particular loop of TBA6 will be better solvated than the corresponding one in TBA−pig. The loop connecting B3 with H3 has residues interacting both with GTP (Asn101) and H5 (Asn102). We believe that this loop has a role both in stabilizing the dimer, by enhancing the interaction with GTP, and in consolidating the GTP-binding site through H bonds with neighboring α-helices. Asn102 is replaced with Cys and His107 with Arg. These modifications do not affect the H bond of Asn101 with the third phosphate group of GTP (1.7 Å), but enhances the interaction with H4 (His107) owing to the new charged residue (Arg), which can make

Table 7. GTP-binding site similarity/dissimilarity index for *Caenorhabditis elegans* α-tubulin family and γ-tubulin (TBG1)[a]

Tubulin	GTP
TBA4	3.71
TBA5	3.97
TBA6	3.57
TBA7	10.85
TBA8	1.98
TBA9	15.83
TBG1	90.29

[a] Entries are in angstroms squared.

multiple H bonds. On the other hand, the H bond with H5 seems to be lost as Cys102 is not a strong proton donor. H4 is 5 residues shorter in TBA6, apparently owing to the replacement of Val159 with Thr. B5 has all hydrophilic residues (Lys166, Glu168, Ser170, and Tyr172) on one side. Lys166 interacts with the H4 backbone, Ser170 with B6, and Tyr172 with H11. In TBA6 the replacement of Ser170 with Ala removes the favorable interaction of B5 with B6. The loop connecting H7 to H8 is exposed to solvent, but has hydrophobic residues (Ala247, Leu248) pointing into solvent, while Arg243 points inside TBA6 toward Asp251, which is at the other end of the loop. H12 contains 16 amino acids, 10 of them being hydrophilic. H12 helps in solvation (Glu420, 429, 433, and Asp424), but also acts as "glue" for the nearby secondary structure units. Arg422 and Met424 interact with H11 and Tyr432 with B10. The last residue in the protein databank file is Val440. We did not extend TBA−pig, but constructed a tail for all *C. elegans* TBA tubulins. The TBA6 tail in our model is made up of an α-helix of 17 (H13) amino acids.

TBA7. TBA7 (Color Plate 6 and Table 6) has one more helix (H11′: Arg445–Tyr451) positioned on the loop that connects H11 with H12. Our analysis of the GTP-binding site in TBA7 shows (Table 7) that this is different from the site found in TBA−pig, and from those in TBA4 to TBA6. We identified three significant changes, i.e., (1) the H bond between the amino group of Asn11 and the carbonyl oxygen of GTP guanine decreases from 2.9 Å in TBA−pig to 2.0 Å; (2) an H bond (1.7 Å) forms between the OH of Thr73 and the second phosphate group of GTP (4.6 Å in TBA−pig); and (3) a change occurs in the H bond between the OH of Tyr210 and one of the OH groups of the GTP sugar (3.1 Å in TBA−pig and 2.7 Å in TBA7).

TBA8. The TBA8 (Table 6) secondary structure has two β-strands more: B1′ (Ile39–Asp40), which is located on the large loop connecting H1 with B2, and B10′ (Glu413–Glu416) on the loop connecting H11 with H12. There is little difference in the length of α-helices between the two proteins. The GTP-binding site is left unchanged by mutations. In fact, this site in TBA8 is the most similar to that in TBA−pig (Table 4) among α-tubulins.

TBA9. TBA9 (Color Plate 7 and Table 6) starts with a segment of 20 amino acids (Met1–Glu20) that is missing in TBA−pig and all other *C. elegans* α-tubulins (Color Plate 1). This segment is modeled as an α-helix (H0 in Color Plate 1) that coils back to the body of the protein and interacts with H4. Similarly, the tail of TBA9 consists of an α-helix (H13) of 11 amino acids (Glu450–Tyr460), which turns toward H8 and H0 and interacts with the loop connecting H8 with B7. For example, the hydroxyl group of Tyr297 makes an H bond with Glu453 (H13). Both TBA9 and TBA−pig have 12 α-helices, but because of inserted (Met1–Phe20, Pro94–Ser108) and deleted (Val323–Met348) amino acid segments the locations of two of the α-helices are different in the two proteins. H2 and H9 (in TBA−pig) are removed and H13 (C-termini) and H0 (N-termini) appear instead. TBA9 has two β-strands more than TBA−pig, B2′ and B2′′ (Color Plate 7), located on the loop connecting B2 with B3. We found four modifications in the GTP-binding site of TBA9; a weak H bond between the amino group of Gln11 and the carbonyl group of GTP guanine (2.9 Å in TBA−pig) is removed completely in TBA9 (4.4 Å), the distance between Thr73 and GTP increases from 4.6 to 6.9 Å, the backbone NH of Ala100 and GTP changes from 3.8 to 5.8 Å, and, finally, the weak H bond between the OH of Thr145 and the second phosphate group of GTP is removed (2.5 to 3.3 Å).

TBG1. Compared with α-tubulin family members TBG1 (Color Plate 8 and Table 6) is very different. First of all, TBG1 has two α-helices (H2 and H8) and two β-strands (B3 and B9) less than TBA−pig. Second, H3 and H5 are longer (by about two and a half times, or more) than their counterparts in TBA−pig. Color Plate 8 shows that TBG1 is definitely less organized than α-tubulin family members; the relative positions of the remaining helices and β-strands change considerably with respect to TBA−pig. We constructed TBG1 as a dimer with TBB1 in order to facilitate the comparison with the α-tubulin family. In our model TBG1 still binds GTP, presumably because TBG1 was derived from the TBA/TBB1 dimer, but it is possible that the monomer conformation of TBG1 will actually hinder the interaction with GTP. In fact the very different structure of TBG1, as compared with the α-tubulin family, seems to indicate this. The Rossmann fold, specific for nucleotide-binding proteins, is substantially distorted in TBG1 and barely resembles the one found in TBA−pig. Nevertheless, the lack of experimental consensus on the ability of TBG1 to bind to a β-tubulin[14,57,58] points to the necessity for further computational investigation.

We conclude the section dedicated to the α-tubulin family and TBG1 with an analysis of the GTP-binding site. We identified 28 H bonds and hydrophobic contacts between 19 amino acids and GTP for the α-tubulin family isotypes. We quantify the differences between the GTP-binding sites in different isotypes with the similarity/dissimilarity index defined above (see Computational Protocol). The data in Table 7 clearly indicate that the GTP-binding site of TBG1 (90.29 Å^2) is very different from that found in the α-tubulin family isotypes. This index is one order of magnitude larger than those calculated for the α-tubulin family isotypes, confirming our earlier conclusion based on the analysis of the secondary structure, that the GTP-binding site in TBG1 may have been lost. TBG1 has 11 of 19 amino acids changed in its GTP-binding site, and the most dramatic is the change of Thr73 to Arg. The proton of the OH group (Thr73) makes an H bond (1.7 Å) with the third phosphate group only in TBA6, while for the other α-tubulin isotypes this distance is in the range of 3.5 Å (TBA5) to 6.9 Å (TBA9). The replacement of Thr73 with Arg brings the amino acid much closer to GTP. Arg73 makes no less than four H bonds (1.7, 1.7, 1.8, and 2.6 Å) both with the second and third phosphate groups of GTP. Another interesting case concerns the role of Tyr210 and Tyr224 in the GTP-binding site. The phenolic oxygen of Tyr224 makes a bridge between the hydroxyl group of GTP sugar and that of Tyr210. In TBG1 both tyrosines are replaced with His (Tyr210) and Phe (Tyr224); thus, two strong H bonds (1.8 Å) are replaced with a weak one (2.8 Å). Among the hydrophobic contacts of GTP with TBG1 we found large deviations for Ala99 (7.8 Å) and Val182 (11.2 Å). The average values for these contacts for the α-tubulin isotypes are 5.5 and 4.0 Å, respectively. The α-tubulin isotypes (TBA4 to TBA9) can be grouped in four sets according to the value of the similarity/dissimilarity index. The closest to TBA−pig is TBA8, with an index of 1.98 Å. TBA4, TBA5, and TBA6 have almost the same index (~3.8 Å), while TBA7 and TBA9 have indices five time larger, i.e., 10.85 and 15.83 Å, respectively. Finally, we conclude that TBA9 has the

most different GTP-binding site with respect to TBA−pig. However, our analysis cannot lead to a straightforward hierarchy of the stability of TBA/TBB dimers based solely on the results given above because such a hierarchy requires thermodynamic data about dimer and monomer stability. However, we speculate that the tubulins with GTP-binding sites very dissimilar to that in TBA−pig have less stable dimers.

Caenorhabditis elegans β-Tubulin Family

β-Tubulins are closely related to α-tubulins,[15] but unlike the α-tubulins β-tubulin isotypes (TBB−pig, TBB1 to TBB6) have very similar secondary and tertiary structures. β-Tubulin isotypes were obtained by altering the 3D structure of TBB−pig in the TBA−pig/TBB−pig dimer followed by optimization with the AMBER force field, hence their conformations are those found in the dimer rather than the monomer form. The presence of GDP- and TXL-binding sites helps in indicating the differences among β-tubulin isotypes. Hence, we used the similarity/dissimilarity index for these two binding sites to quantify the resemblance among the members of the β-tubulin family.

TBB−pig. TBB−pig (Color Plate 9) is 5 amino acids shorter than TBA−pig and has one additional α-helix and β-strand.[15] H8 and H12 (TBB−pig) are missing from TBA−pig, and H2 (TBA−pig) is not in TBB−pig. In addition, B2 and B3 (TBB−pig) are missing in TBA−pig, and B3 (TBA−pig) is not in TBB−pig. Note that in our labeling scheme B2 of TBA−pig corresponds to B4 of TBB−pig. The α-helices and β-strands in the α- and β-monomers have about the same length except for H1 and H12 (TBA−pig), which are about 4 residues shorter than their corresponding counterparts in TBB−pig (H1 and H13). Next we analyze the differences between TBB−pig and the other members of β-tubulin family.

TBB1. In TBB1 (Table 6) the mutated amino acids are not located near the ligands that bind to TBB (GTP, GDP, and TXL) except for Gly277 ⇒ Ser and Ser278 ⇒ Asn, which are close to the TXL-binding site. TBB1 has two β-strands (B2 and B3) and one α-helix (H12) less than TBB−pig. According to our similarity/dissimilarity index for GTP/GDP/TXL-binding sites (Table 8), TBB1 is closest to TBB−pig among all β-tubulin isotypes.

TBB2. TBB2 (Color Plate 10 and Table 6) also lacks B2 and B3; instead it has another β-strand (B8′) not present in TBB−pig. In TBB2 one mutated residue, Ala231 ⇒ Met, is close to the TXL-binding site. While the GTP/GDP-binding sites remain basically unaltered, there are a few changes in the TXL-binding site. For example, the H bond between the OH of Thr247 and one of the OHs of TXL (on the four-member ring) increases from 2.0 to 3.3 Å, and the H bond between the NH_2 of Arg276 and the OH of TXL (on the six-member ring) increases from 2.6 to 3.0 Å. The similarity/dissimilarity index for TBB2 is 5.86 as compared with 1.81 for TBB1 (Table 8), which shows that, as far as the TXL-binding site is concerned, TBB2 is less similar to TBB−pig than TBB1.

Table 8. GTP-, GDP-, and TXL-binding sites similarity/dissimilarity indexes for *Caenorhabditis elegans* β-tubulin family[a]

Protein	GTP	GDP	TXL	GTP + GDP + TXL
TBB1	0.05	0.08	1.68	1.81
TBB2	0.08	0.11	5.67	5.86
TBB3	3.89	0.09	36.58	40.56
TBB4	0.06	0.12	10.88	11.06
TBB5	0.06	0.09	29.81	29.96
TBB6	147.63	8.48	73.71	229.82

[a] Entries are in angstroms squared.

TBB3. In TBB3 (Color Plate 11 and Table 6) there are two mutations in the TXL-binding site: Thr274 ⇒ Ser and binding site Arg276 ⇒ Lys. Unlike TBB1 and TBB2, in TBB3 GTP is slightly altered (similarity/dissimilarity index of 3.89). We found three modifications in the GTP-binding site map, i.e., the H bond between NH (Leu246, 1.8 Å in TBB−pig) and the first phosphate group of GTP is removed (3.3 Å) while another H bond is made with the nitrogen of the five-member ring of guanine (1.9 Å). Also, the H bond between the RNH_3^+ group of Lys350 and the carbonyl group of Asn247 (1.7 Å) is weakened (2.4 Å). In addition, in the TXL-binding site there are 10 H bonds/hydrophobic contacts that are altered from their values in TBB−pig, which make the similarity/dissimilarity index quite large (36.58). The largest changes are the loss of the H bond between the OH of Ser234 and the backbone carbonyl of Ser230 (2.0 ⇒ 3.4 Å), the loss of the H bond between the OH of Thr247 and the OH of the four-member ring of TXL (2.0 ⇒ 4.6 Å), and the loss of the two H bonds of the NH_2 group of Gln279 with the carbonyl of the eight-member ring (2.6 ⇒ 5.7 Å) and that of the OH of the six-member ring (2.8 ⇒ 5.5 Å) of TXL. TBB3 has an 18-residue long α-helix tail (H14) nearly parallel to H13.

TBB4. TBB4 (Table 6) has a 19-residue long α-helix tail nearly perpendicular to H13. TBB4 has the same two mutations (Thr274 ⇒ Ser, Arg276 ⇒ Lys) in the TXL-binding site as TBB3, but its similarity/dissimilarity index is more than three times smaller than the TBB3 index. Unlike TBB3, in TBB4 the GTP-binding site is not altered from its configuration found in TBB−pig, but there are seven modifications in the network of H-bond/hydrophobic contacts in the site. Four of them are similar to those found in TBB3. In addition, we note the loss of the H bond between one NH_2 group of Arg276 with the carbonyl of the TXL benzoxy group (1.8 ⇒ 3.2 Å).

TBB5. As with TBB3 and TBB4, TBB5 (Table 6) has the same two mutations in the TXL-binding site, but it lacks the B2 and B3 β-strands. The similarity/dissimilarity index of TBB5 (11.06) is between that of TBB2 and TBB4. TBB5 has no change in the GTP-binding site, while there are nine changes in the TXL-binding site. Many of these changes are also found in TBB2 through TBB4; thus we mention here only the formation of an H bond between the NH_2 of Gln279 and the OH of the six-member ring of TXL (2.8 ⇒ 1.9 Å), and the increase of the former H bond between the NH_2 of Arg276 and the same OH (2.6 Å in TBB−pig) from 4.1 Å (TBB3) to 5.4 Å.

TBB6. The last member of β-tubulin family, TBB6 (Color Plate 12 and Table 6), is much shorter than the other isotypes. The deleted residues in TBB6 are grouped into five sequences of 5, 2, 1, 3, and 14 amino acids. Compared with other members of the β-tubulin family, TBB6 has the most dramatic modifications in all three binding sites. The GTP-binding site has three altered residues: Asn247 ⇒ Lys, Asp327 ⇒ Ala, and Lys350 ⇒ Asn. The GDP-binding site has one residue deleted (Asn226) and four mutations: Val169 ⇒ Ile, Leu207 ⇒ Ile,

Tyr222 ⇒ Thr, and Val229 ⇒ Ala. The TXL-binding site has one deletion (His227) and six mutations: Asp26 ⇒ Glu, Cys239 ⇒ Phe, Thr274 ⇒ Tyr, Arg276 ⇒ Lys, Gln280 ⇒ Ile, and Pro358 ⇒ Ser. The similarity/dissimilarity index for TBB6 (229.82) is one order of magnitude larger than that for TBB5. Because in TBB6 Asp272 is replaced with Ala, the distance between this residue and the OH group of the GTP sugar unit increases from 4.1 to 6.2 Å. Similarly, the replacement of Lys350 with Asn removes the two H bonds between RNH_3^+ and the first phosphate group of GTP (1.7 ⇒ 10.2 Å, 2.6 ⇒ 10.7 Å). In addition, the H bond between RNH_3^+ and the carbonyl group of Asn247 is also removed (1.7 ⇒ 4.0 Å). In the GDP-binding site we detected three changes: an H bond is formed between the NH_2 of Gln15 and the nitrogen from the six-member ring of guanine (3.1 ⇒ 1.9 Å) and by deleting Asn222, two H bonds between the carbonyl and amino moieties of the $CONH_2$ group and NH and CO of guanine are removed. Finally, we note that there are 14 changes in the TXL-binding site. Among the most drastic modifications we mention the loss of the H bond between the nitrogen of His227 and the HO of TXL (by deleting the His residue) and the loss of two other H bonds, between the OH of Thr274 and the OH of the four-member ring in TXL (by changing Thr274 to Tyr).

Overall, Table 8 shows that, as far as the GTP-binding site is concerned, TBB1, TBB2, TBB4, and TBB5 are close to TBB−pig, TBB3 is slightly different, while that of TBB6 is dramatically altered. This was expected because of the many changes in the TBB6 primary structure. Our similarity/dissimilarity index can quantify these differences and shows that there is a large change in the GTP-binding site of TBB6. Our map for the GDP-binding site includes 20 H bonds and hydrophobic contacts, and the data in Table 8 show that there is almost no difference between TBB1, TBB2, TBB3, TBB4, TBB5, and TBB−pig. TBB6 has important changes in this map. Table 8 also shows that the TXL-binding site is more sensitive to changes in the primary structure than the GTP- and GDP-binding sites. Moreover, in light of these results the experimental observation that TXL induces tubulin polymerization in the absence of GTP or MAPs[59,60] deserves further investigation. Finally, we conclude that TBB6 is unusual among the members of the β-tubulin family, and that the stability of an α/β-dimer made with TBB6 must be seriously affected.

CONCLUSIONS

We described the genetic structure (including regulatory sequences) for the entire *C. elegans* tubulin family, consisting of 16 genes (*tba-1* to *tba-9*, *tbb-1* to *tbb-6*, and *tbg1*). *Caenorhabditis elegans* tubulin isotypes share high homology with mammalian tubulins. The publication of the X-ray crystal structure (at a resolution of 3.7 Å) of pig brain α/β-tubulin dimer by Nogales et al.[15] inspired us to carry this computational study to the 3D structure of all *C. elegans* α-, β-, and γ-tubulin isotypes. Although the X-ray resolution of the crystal structure is low, we still pursued our computational study in order to show that it is possible to explore the structure of related proteins with some degree of confidence by using current state-of-the-art computer modeling software (AMBER, MIDAS, WebLab Viewer). We suggest caution in the use of this computational strategy for obtaining definitive answers about protein structure, but we also note its usefulness in comparative studies of protein families and in delineating trends in stability and reactivity among the members of the same family. We believe that at present this is the only computational technique that can be used to study the 3D structure of large proteins with no X-ray crystal structure. The pace of sequencing proteins and mapping out of the genetic code is far faster than that of obtaining X-ray structures, and thus the only hope for the moment is to model structures by computational means and in this way to obtain information about their reactivity.

Our analysis of the secondary and tertiary structure of the *C. elegans* tubulins shows that there is a high degree of resemblance among these proteins, as obtained by computation. The crystal structure of these proteins may differ from their computationally derived structure because of the assumed homology, but according to our statistical analysis there is little difference between the crystal and optimized structures (TBAB-pig). Overall, β-tubulins have one more α-helix and β-strand than do α-tubulins, but the size patterns of secondary structure units in α- and β-tubulins are similar, with a few exceptions: e.g., H2 is much shorter and H12 is much longer in α-tubulins relative to β-tubulins. We believe that there are fine differences in their tertiary structure that differentiate the members of the tubulin family. We have analyzed these subtle differences in terms of changes in the GTP-, GDP-, and TXL-binding sites. We mapped out these binding sites by making an inventory of the H bonds and hydrophobic contacts of these ligands with the nearby amino acids and quantified the differences with a similarity/dissimilarity index, which accounts for the deviation of these distances from their corresponding values found in the TBA−pig/TBB−pig dimer. According to this index evaluated for the GTP-binding site we conclude that TBA7 and TBA9 are less related to TBA−pig than are TBA4, TBA5, TBA6, and TBA8, while TBB3, TBB4, and TBB5 are less related to TBB−pig than are TBB1 and TBB2. On the other hand, TBG1 is an unusual case in the *C. elegans* tubulin family, and there is much debate in the literature on the GTP-binding capability of TBG1.[14,57,58] One of the issues that we tried to address with our computational analysis of the tertiary structure of the *C. elegans* tubulins is the relative stability of TBA/TBB dimers. In the absence of any thermodynamic data on dimer stability (e.g., binding constants), we assume that TBA−pig/TBB−pig is the most stable dimer and take it as a reference for assessing the stability of dimers made with the *C. elegans* isotypes. Thus, we speculate, according to our similarity/dissimilarity index, that TBA7, TBA9, TBB3, TBB4, TBB5, and TBB6 give dimers less stable than the TBA−pig/TBB−pig dimer. Both experimental and further computational investigations are underway in our groups to test these predictions on tubulin dimer stability in *C. elegans.*

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