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Review

A structural view of microtubule dynamics

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Abstract. The essential microtubule property of dynamic instability is based on the binding, hydrolysis and exchange of GTP in each tubulin dimer. The recent high-resolution structures of tubulin and the microtubule have given us the first view at atomic level of properties such as nucleotide exchangeability, the linkage between polymerization and nucleotide hydrolysis, and the origin of microtubule destabilization, as well as the mode of action of antimitotic agents such as taxol.

Key words. Tubulin; microtubule; dynamic instability; nucleotide; structure; electron crystallography.

Introduction

Microtubules are cytoskeletal polymers essential for the survival of all eukaryotes. The functions of microtubules include cell transport, cell motility and mitosis, and involve the interaction of microtubules with a large number of microtubule-associated proteins (MAPs) [1]. The role of MAPs includes the regulation of microtubule stability, the formation of microtubule networks and the transport of cargos along microtubules [1, 2].

Microtubules are made of repeating α,β-tubulin heterodimers. Tubulin dimers bind head to tail to form linear protofilaments, and about 13 protofilaments associate in parallel to make the microtubule wall. The resulting polymer has a defined polarity, with two ends that are structurally and functionally distinct. In the cell the minus end is often anchored at microtubule organizing centers (MTOCs), whereas the plus end is free in the cytoplasm or attached to a specific target such as the kinetochore [3].

Essential to the function of microtubules is their dynamic character [4]. More than 20 years ago it was shown that microtubules can exist in a steady state of unidirectional flux in which subunits move through the polymer as a result of net polymerization at one end and net depolymerization at the other [5], a property known as treadmilling. Although treadmilling was initially thought as being relevant only as an in vitro property, recent results have shown its importance in the cell, both for interface and mitosis microtubule behavior [6]. A more general property of microtubules is to switch stochastically between growing and shrinking phases, both in vivo and in vitro, a phenomenon known as dynamic instability [7]. More than 10 years of biochemical studies have shown that the dynamic properties of microtubules have their origin in the binding and hydrolysis of guanosine 5′-triphosphate (GTP) by tubulin. Each tubulin monomer binds one molecule of GTP. The nucleotide bound to α-tubulin, at the so-called N site, is nonexchangeable, whereas the nucleotide bound to β-tubulin, at the E site, is exchangeable. GTP is required at the E site in order for tubulin to polymerize [8]. Closely following polymerization, the nucleotide at the E site is hydrolyzed and becomes nonexchangeable. The result is that the body of the microtubule is made of guanosine 5′-diphosphate (GDP)-tubulin subunits that energetically favor depoly-

merization. The standing model for dynamic instability, known as the GTP cap model [7], is that the microtubule structure is stabilized by a layer of GTP-tubulin subunits at the ends that still retain their GTP. When this cap is lost, the microtubule rapidly depolymerizes. The model is supported by studies using nonhydrolyzable GTP analogues and showing that, although microtubules polymerize, they lose their dynamic properties [9].

Although dynamic instability is inherent even in purified tubulin solutions, microtubules are more dynamic in vivo, with markedly different behaviors at different stages in the cell cycle [4, 10, 11]. Interphase microtubules are fairly stable, whereas mitotic microtubules are very dynamic and have a very fast turnover rate [12]. Among the factors that regulate dynamic instability are stabilizers such as classical MAPs (e.g. MAP4 and tau) [1], and destabilizers like stathmin, katanin and the Kin I kinesins [13–15]. While studies of new cellular microtubule regulators are beginning to show us how the cell controls the microtubule cytoskeleton [13–15], the recent structures of the tubulin dimer [16] and the microtubule [17] are giving us a molecular insight into the origins of the inherent self-regulatory properties of microtubules.

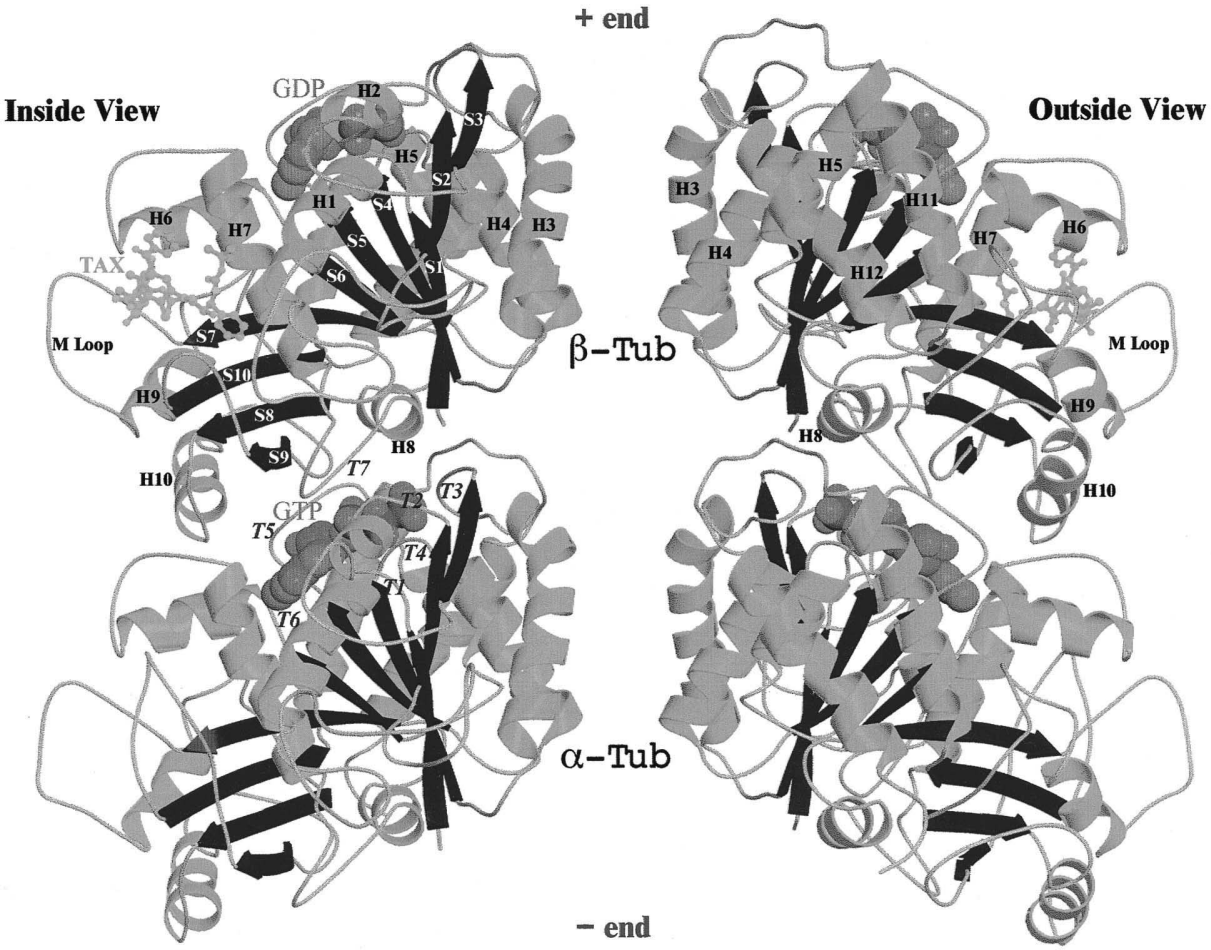


Figure 1. Structure of the tubulin dimer. Ribbon diagram of the crystal structure of the α,β-tubulin dimer from zinc-induced tubulin sheets stabilized with taxol, obtained by electron crystallography to 3.7-Å resolution. The orientation of the dimer is such that the plus end of the microtubule is towards the top and the minus end towards the bottom of the page. Two complementary views, corresponding approximately to the inside and outside views in a microtubule, are shown. The different secondary structure elements are labeled on the top subunit (β-tubulin), as are the loops involved in nucleotide binding (the labeled loops are those around the nonexchangeable GTP in the α subunit).

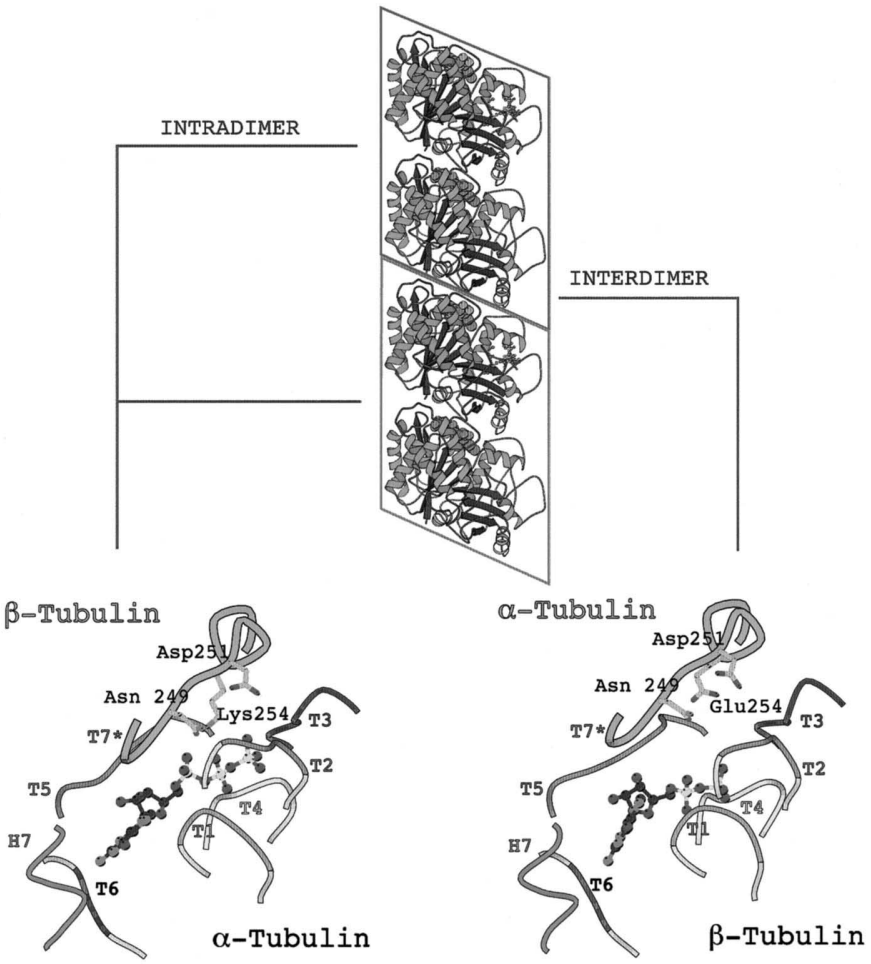


Figure 2. Burial of the nucleotide at longitudinal monomer-monomer interfaces. Protofilament showing the burial of the nucleotide at intradimer and interdimer interfaces. The loops involved in direct contact with the nucleotide, within the same subunit (T1–T6) and in the subunit across the interface (T7) are shown for the contacts between monomers at the intradimer (left) and interdimer (right) contacts. Essential conserved residues in loop T7 are also shown.

Structure of the tubulin dimer: nucleotide binding and exchangeability

The structure of the tubulin dimer was obtained by electron crystallography of zinc-induced tubulin sheets [16]. These sheets, discovered in the 1970s, are formed by the antiparallel association of protofilaments [18]. The resulting polymer has no polarity and no overall curvature, so that it can grow in two dimensions, making what can be considered a two-dimensional crystal. Addition of taxol stabilizes the sheets against cold temperature depolymerization and aging [19]. Using low-dose methods, cryopreservation, and image processing, a structure of the tubulin dimer bound to taxol was obtained at 3.7-Å resolution. The crystallographic model of tubulin contains all but the last 10 residues of α-tubulin and the last 18 residues of β-tubulin. These C-terminal tails are very acidic and were expected to be highly disordered [20]. Figure 1 shows a ribbon diagram of the structure of the $\alpha\beta$-tubulin dimer. Each monomer is very compact, formed by the tight interaction of three sequential domains that are functionally distinct. The N-terminal, nucleotide binding domain is formed by six parallel β strands (S1–S6) alternating with helices (H1–H6). Each of the loops that join the end of a strand with the beginning of the next helix are directly involved in binding the nucleotide (loops T1–T6) (fig. 2). Within each subunit, nucleotide binding is completed by interaction with the N-terminal end of the core helix H7. The core helix connects the nucleotide binding domain with the smaller, second domain, formed by three helices (H8–H10) and a mixed β sheet (S7–S10). The C-terminal region is formed by two antiparallel helices (H11–H12) that cross over the previous two domains.

The position of the nucleotides in the α and β subunits

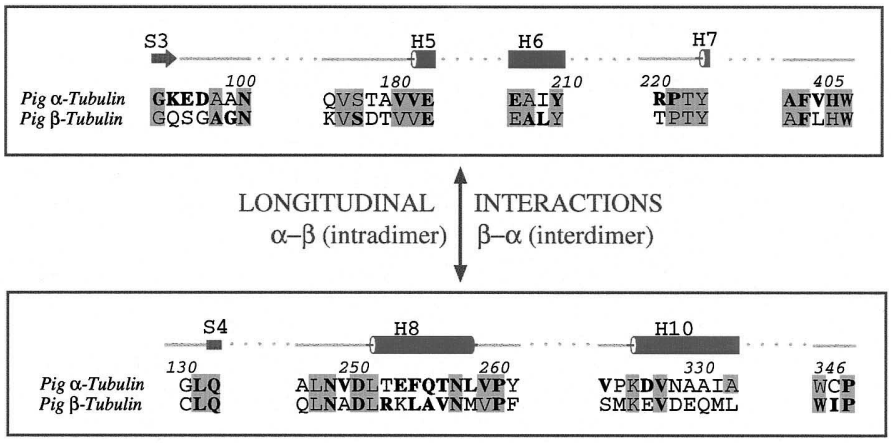


Figure 3. Residues involved in longitudinal contacts between tubulin monomers. Sequences in α- and β-tubulin containing the residues directly involved in longitudinal contacts between tubulin subunits, and their corresponding secondary structure. Intradimer contacts are made between the α-tubulin residues in the upper box, and the β-tubulin residues in the lower box. Alternatively, interdimer contacts are made between the β-tubulin residues in the upper box and the α-tubulin residues in the lower box. Residues totally conserved within the α- or β-tubulin family are shown in bold (conservation from [40]). Those residues that are the same in both α- and β-tubulin sequences are boxed in gray.

is identical with respect to the monomer boundaries, but very different with respect to the dimer boundaries. The nucleotide in the α subunit (GTP) is buried at the monomer-monomer interface within the dimer, explaining the nonexchangeability of the site. On the other hand, the nucleotide at the E site is partially exposed on the surface of the dimer, allowing its exchange with the solution.

Protofilament structure and nucleotide hydrolysis

Because the structure of tubulin was obtained from a polymerized form of the protein, the crystallographic model includes the whole structure of the protofilament as well as information on the longitudinal interactions between dimers. The longitudinal contacts between dimers are very similar to those between monomers within the dimer, thus polymerization involves the burial of the E-site nucleotide and its loss of exchangeability. In both the intra- and interdimer interfaces, the nucleotide is directly involved in the contact between subunits (fig. 2). There is, thus, a region in the tubulin structure involved in the interaction with the nucleotide in the next subunit along the protofilament. This region includes the loop connecting the core helix (H7) with the first helix of the second domain, H8 [21]. This loop, named T7, includes highly conserved residues in both tubulin subunits. The conservation of these residues within tubulins and with FtsZ requires special mention.

FtsZ is a ubiquitous protein in eubacteria and archaebacteria that is essential for bacterial cell division [22]. FtsZ localizes at the site of septation during cell division [23] and was identified by mutations that interfere with cytokinesis [24]. FtsZ binds and hydrolyzes GTP [25, 26], forms filaments in vitro which are reminiscent of tubulin protofilaments [27], and shows a higher degree of sequence identity with tubulin than with any other protein in the database. Although this degree of identity is only about 10%, it includes a glycine-reach segment that is considered the tubulin signature motif [28]. These similarities led to the hypothesis that tubulin and FtsZ could be structurally related [28]. This was emphatically confirmed when the crystal structures of both proteins were obtained [16, 29] and later compared in detail [21]. The structural superposition of both proteins showed that the conserved residues are all localized to sites of interaction with the nucleotide, loops T1–T6 and helix H7, as well as T7. This leads to a model of FtsZ polymerization in which T7 contributes to the interaction with the nucleotide in the next molecule along the filament. The residues conserved in T7 include Asn249 and Asp251 (numbers from the tubulin sequences), which are totally conserved in all α- and β-tubulins, all FtsZs and all γ-tubulins except that in *Saccharomyces cerevisiae*. These residues in tubulin are involved in the binding of the α,β-phosphates of the nucleotide of the next subunit. The interaction with the nucleotide across the longitudinal interface is completed by Lys254 in β-tubulin (within the H8 helix), which interacts with the γ-phosphate of the N-site nucleotide, and in α-tubulin by Glu254, which is in a position that would be close to the γ-phosphate of the E-site nucleotide (in the crystal structure this site is occupied by GDP due to hydrolysis during the formation of the sheets). When the equiva-

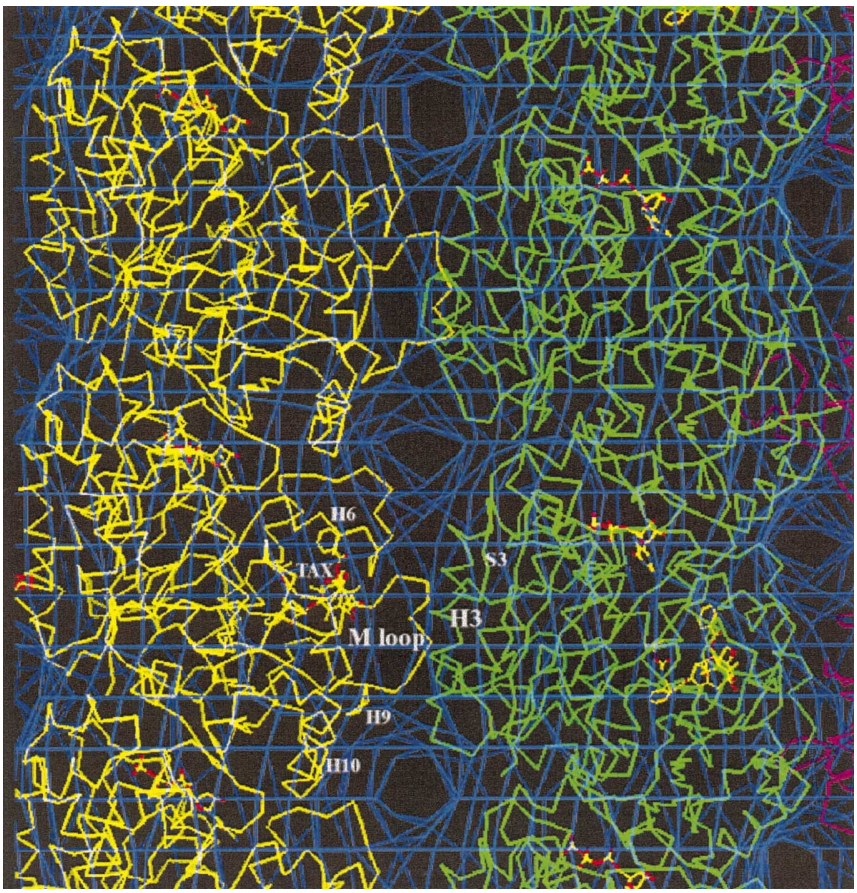
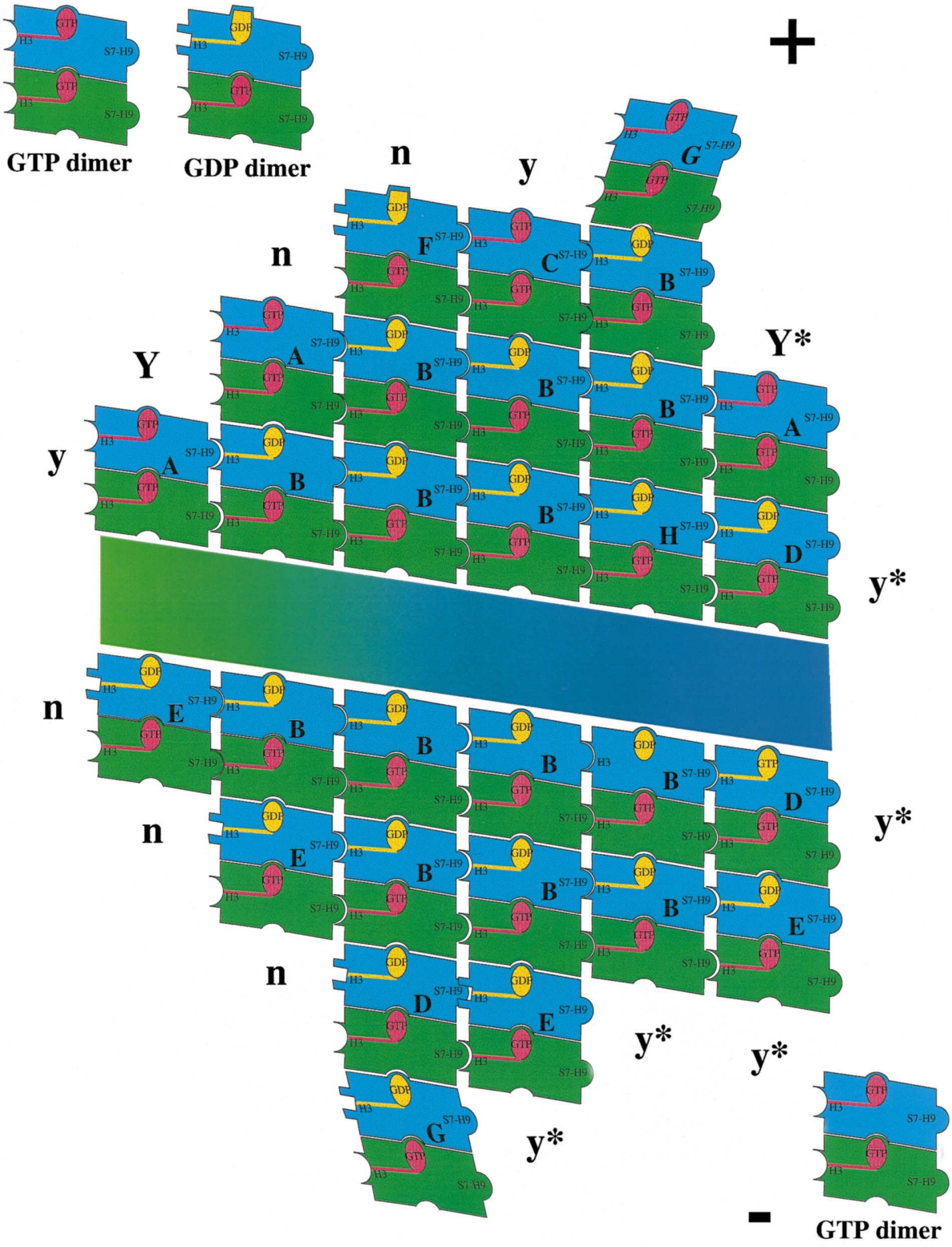


Figure 4. Microtubule docking and lateral contacts. Fitting of the crystal structure of the protofilament into a 20-Å reconstruction of the microtubule. The blue mesh corresponds to the low-resolution microtubule map. Part of two protofilaments is shown, corresponding to a view from the outside of the microtubule, with the plus end at the top of the figure. The crystal structure of the two protofilaments is shown in a Cα carbon display. Structural elements involved in the lateral contact between protofilaments are indicated.

lent residue in FtsZ, an aspartic acid, is mutated to alanine, the binding of GTP is not affected, but its hydrolysis is totally abolished [30]. The position of α:Glu254 in the crystal structure of the protofilament, together with the results of mutagenesis in the equivalent residue in FtsZ, strongly supports the idea that this residue is essential for the activation of hydrolysis accompanying tubulin polymerization. The idea that tubulin is its own GAP (GTPase activating protein), based on the linkage of hydrolysis with polymerization, is thus supported and extended with the identification of an activating region in the molecule that comprises mainly loop T7 and helix H8 [21].

The longitudinal contact between subunits is very extensive [17, 21]. The two surfaces at the interface are convoluted in shape and highly complementary. About 52% of the residues are totally conserved across species for the intradimer interface, whereas about 40% are conserved at the interdimer contact (fig. 3). About 3000 Å^2 of the protein surface is buried with the formation of the dimer from the subunits, or in a contact between dimers. Apart from a very important van der Waals contribution, the character of the interface is mainly hydrophobic and polar, with minimal electrostatic interactions [17]. One salt bridge seems clear in the crystal structure, between β:Glu253 and α:Glu98, at the intradimer interface. In a dimer-dimer contact these residues are substituted by α:Thr253 and β:Gly98 [17]. The existence of this extra ionic interaction may be essential for the specificity of the intradimer contact and/or its strength.

The position of the E-site nucleotide at the dimer-dimer interface suggests that the nucleotide state should have a direct influence on longitudinal interactions by modifying the geometry and chemical properties of the interacting surfaces, although additional allosteric effects affecting the overall conformation of


GTP dimer
GDP dimer
+
-
n
y
Y
Y*
y*
n*
GTP dimer

the subunits are also possible (see later). Electron microscopy studies showing a change in the axial repeat of GMPCPP microtubules with respect to regular GDP microtubules had been interpreted as a change in the tubulin monomer length [31].

Microtubule structure and lateral contacts

Although the crystal structure of zinc sheets allows us to characterize the longitudinal contacts between tubulin subunits along a protofilament, it does not contain any information about the lateral interaction between protofilaments in a microtubule. In order to identify the elements involved in lateral contacts, and to position the different structural elements of tubulin in the context of the microtubule, it was necessary to obtain an atomic model of the whole polymer. This was recently achieved by docking the high-resolution model of the protofilament into a 20-Å reconstruction of the microtubule obtained by cryoelectron microscopy and image analysis of frozen-hydrated microtubules [17]. The three-dimensional structure of the microtubule was calculated using helical reconstruction methods on selected microtubules containing 15 protofilaments and a four-start helical lattice. The docking was done both manually and analytically by maximizing the correlation coefficient between the low-resolution map and a density generated from the atomic model of the microtubule. The docking is unique and shows an impressive agreement between the two structures, testifying to the conservation of the tubulin conformation in microtubules and two-dimensional sheets (fig. 4).

The docking indicates that the lateral contact is dominated by the interaction of the M loop, the loop between S7 and H9, with loop H1–S2 and helix H3 (fig. 4). This interaction, in comparison with the longitudinal contact, has an important ionic contribution, both for α–α and β–β contacts. In β-tubulin the M loop is an essential part of the taxol binding pocket, whereas H3 follows loop T3, which is involved in binding the γ-phosphate of the E-site nucleotide. The conformation of the M loop is stabilized in the α subunit by the long S9–S10 loop. In the β subunit a similar effect may be the cause of the stabilizing effect of taxol and taxol-like compounds. On the other hand, the destabilizing effect of nucleotide hydrolysis may be due to a conformational change transmitted to H3 through the γ-phosphate sensing loop.

The M loop is in a position where it could hinge without disrupting its interaction with the adjacent subunit, and therefore allow for the known variability in protofilament number of reconstituted microtubules. The sequence of this loop corresponds to one of the most divergent segments between α and β tubulins. This difference must be essential in selecting the type of microtubule lattice. It has been proven, by using motor decoration of microtubules [32], that the B lattice is the predominant arrangement in microtubules, where lateral contacts are made between homologous subunits, i.e. α–α and β–β. In most microtubules, however, there is a 'seam' in which the contact is made between heterologous subunits, i.e. α–β and β–α. These types of contacts could have a substantially different energy, given the difference in the M loop of α and β subunits. A possibility to be considered is that this less favorable type of contact occurs during the closure of the observed sheets at the ends of growing microtubules [33]. The cooperative effect of many of these lateral contacts occurring simultaneously may compensate for a less favorable energy of contact for each individual tubulin subunit. The seam may play an important role in microtubule stability and/or binding to specific MAPs.

The docking shows that the C-terminal helices form the crest of the protofilaments on the outside surface of the microtubule. Helices H11 and H12 are, therefore, likely candidates for the binding of motor proteins of the kinesin families, based on cryoelectron microscopy reconstructions of microtubules decorated with motors. The bumpy inside surface of the microtubule is defined by a series of loops, especially loops H1–S2 and H2–

Figure 5. Microtubule end schematic. Proposed model of the structure and properties of microtubule ends. The type of lattice position and nucleotide state of each dimer are indicated by a letter (from A to G); the type of position available for a new addition is labeled to indicate whether the addition is or is not favored. Notice that only GTP dimers can add to the minus end (where they are soon hydrolyzed), whereas GDP dimers can bind to most of the allowed addition sites at the plus end. The schematic is an oversimplification; it does not, for example, take into account the possibility of a seam. A seam will give rise to a larger number of possibilities and may be essential in the process of dynamic instability, especially to explain the frequency of rescue and catastrophe. Tubulin dimers with different lattice positions are labeled as follows: A, freshly added GTP dimers at the plus end, in which GTP has not been hydrolyzed. Their arrival has caused the hydrolysis of the nucleotide on the dimer below; B, the majority of tubulin subunits in the microtubule are in this position. The nucleotide has been hydrolyzed but the subunit is buried within the lattice; C, this subunit is not yet hydrolyzed but has made lateral contacts at both sides; D, these subunits are buried except for a lateral contact; E, these are GDP dimers at the minus end that have only made one longitudinal and one lateral contact; F, this is an exposed GDP subunit at the plus end. It could have been generated by addition of a GDP dimer, by exchange of GTP for GDP, or by loss of an end dimer after hydrolysis has been catalyzed; G, depolymerizing dimers. They are kinked at an unfavorable longitudinal contact and are bent towards the viewer. Addition of a GTP dimer is favored at all the positions marked 'y' or 'y*', with 'Y' and 'Y*' being the most favorable. It is disfavored in all of those marked '*n*' because of poor lateral or longitudinal contacts. Addition of GDP dimers is only possible at positions marked 'y' and 'Y'.

S3, which were poorly resolved in the crystal structure of the sheets.

Taxol binding site and mode of action

Another loop defining the inside surface is the S9–S10 loop. This loop is eight residues longer in α-tubulin due to an insertion. The position of these extra residues in α-tubulin is occupied by taxol in the β-tubulin subunit, suggesting both that the action of taxol could be to mimic the α-tubulin insertion, and furthermore, that endogenous peptides could bind to the taxol binding pocket in the cell to regulate the stability of the microtubule. But how does taxol work? The position of taxol by the M loop agrees with the effect of taxol on microtubule protofilament number (reduction from an average of 13 to an average of 12) [34], and suggests that the action of taxol could be the stabilization of lateral contacts by affecting the stability/conformation of the M loop. The polymerization of GDP-tubulin in the presence of taxol [35] could then be understood as a change in the M loop that compensates for the unfavorable conformation of H3 in the absence of the γ-phosphate [17]. On the other hand, the taxol binding pocket comprises elements from different domains, so that taxol could act as a bridge that maintains a certain orientation between the nucleotide-binding domain and the second domain. Such a model has been proposed based on the comparison of the structures of tubulin and FtsZ and on the constraints within the interdimer interface of loop T3, which links the γ-phosphate region to H3 [36]. In this model forces due to the loss of the γ-phosphate would be transmitted to the base of the nucleotide and to the core helix (H7), therefore changing the relative orientation of the nucleotide domain and the second domain of tubulin and FtsZ. Such a change in orientation could affect both lateral and longitudinal interactions, and the taxol effect would be to counteract this mechanism. Recent studies on the depolymerizing effect of XKCM1 on taxol- versus GMPCPP-stabilized microtubules [37] do not seem compatible with an effect of the drug on the stability of longitudinal contacts, but the data are somewhat indirect.

The taxol binding site is on the inside surface of the microtubule. Binding experiments of a fluorescent taxol derivative have shown that binding to preformed microtubules is extremely fast and cannot be explained by a simple diffusion mechanism from the microtubule ends [38, 39]. Although the microtubule wall has holes that could allow taxol to pass through them, the holes are too small for the large derivative. Furthermore, this tight passage does not seem compatible with the observed kinetic parameters of binding [J. M. Andreu and F. Diaz, personal communication]. Additional models include the passage through lattice defects, or the fast 'breathing' (opening and closing) of the microtubule lattice [39]. A breathing mechanism could be related to the existence of seams in the microtubule lattice. Experiments with hyperstable microtubules, such as those in axonemes, may shed some light on this problem.

Microtubule ends and dynamic instability

In the high-resolution model of the microtubule the orientation of the protofilaments is such that the plus end is crowned by β-tubulin subunits exposing their nucleotide ends to the solution, whereas the minus end is crowned by α-subunits exposing their catalytic end. This orientation has very important repercussions for the GTP-cap model. When a dimer is added to a plus end, its catalytic end contacts the E-site nucleotide of the previous subunit forming the interface that should bring about hydrolysis. However, the GTP in the E-site of the newly added tubulin dimer will not be hydrolyzed until the next subunit is added. The result is that the plus end will generally have a GTP cap of at least one tubulin monolayer. The situation is very different at the minus end. When a new dimer arrives, contact is made between its E-site nucleotide and the catalytic region of the last subunit at the end. Therefore, in general, there will be no GTP cap at the minus end. A GTP cap could be generated, however, if the rate of dimer addition were faster than that of GTP hydrolysis or Pi release. Such fast addition would also increase the size of the cap at the plus end. The disappearance of the cap at the plus end, in turn, could occur by exchange of the E-site GTP for GDP, by addition of GDP subunits, or by detachment of GTP subunits, therefore exposing the already hydrolyzed dimers.

Based on the existing structure, it seems reasonable to assume that the effect of hydrolysis on lateral contacts is mostly limited to the β subunit containing the hydrolyzable nucleotide at the E site. This establishes further differences between the plus and minus ends. It is reasonable to think that the stability of a microtubule end could be mainly determined by the lateral interactions at the very last monomer, with the second monomer having a smaller additional effect. The last lateral contact at the minus end is always made by an α subunit (containing the invariable N site), and is likely to be insensitive to the nucleotide state in the β subunit. We hypothesize that the lateral contacts between α-tubulins will be strong. This is based on the fact that the nucleotide content of α-tubulin is always

GTP, and on our proposition that the extra eight residues in α-tubulin within the S9–S10 loop mimic in this subunit the effect that taxol has in the β subunit, namely the stabilization of the M loop. According to this hypothesis, the minus end should be fairly stable with respect to lateral interactions (even in the absence of a GTP cap), with a strong last contact between α subunits. In contrast, the plus end could be in one of two very different states concerning lateral interactions, depending on whether the contacts at the very last monomer are between β subunits containing GTP (strong contacts) or GDP (weak contacts). This results in two very different dynamic behaviors for the plus and minus ends that rely on tubulin being a dimer where only one monomer is regulated by nucleotide hydrolysis.

Figure 5 shows a simplified model of the microtubule plus and minus ends that puts forward some of the simple ideas we have proposed to relate polymerization, nucleotide hydrolysis and dimer stability within the microtubule lattice. The cartoon corresponds to a state of slow polymerization in which hydrolysis is faster than dimer addition. α-Tubulins are green; β-tubulins are blue. At the minus end, hydrolysis of the nucleotide in the incoming dimer occurs at the same time as dimer addition, whereas hydrolysis at the plus end is delayed until the next longitudinal addition takes place. Therefore, in conditions where GTP and GTP-tubulin are abundant, the plus end will have a minimum GTP cap of n subunits, where n is the number of protofilaments. This cap would be larger if hydrolysis and/or Pi release were slow compared with the rate of subunit addition. The cap could become smaller by addition of GDP subunits, exchange of GTP for GDP, or detachment of an end subunit after it has hydrolyzed the nucleotide in the preceding subunit. At the minus end a GTP cap can only exist if hydrolysis and/or Pi release are slow with respect to subunit addition. Under the conditions assumed for this cartoon (slow tubulin addition), the minus end should be slowly depolymerizing.

Conclusions

The high-resolution structures of the tubulin dimer and the microtubule suggest some of the molecular bases for properties essential to dynamic instability, such as the linkage between polymerization and GTP hydrolysis. The regions in the tubulin structure involved in polymerization contacts have been identified, and structure-based models have been proposed for the stabilizing action of taxol and the destabilizing action of GTP hydrolysis. However, our information is restricted to a single conformational state of tubulin and to sensible extrapolations from it. Direct structural information for other functional states is still required. Both structural and functional information is lacking concerning the regulation of microtubule dynamics in the cell by already identified regulatory factors. Furthermore, many such regulatory factors may remain undiscovered. The structure of tubulin will prove a precious tool for the inspiration and progress of new experiments that will one day give us a more comprehensive understanding of the exquisite details in the regulation of the microtubule cytoskeleton.

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