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Microtubule structure and its stabilisation

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Microtubules are designed to be dynamically unstable. GTP hydrolysis converts an initially stable polymeric structure into an unstable one in which strain at the interfaces between longitudinal neighbours in the helical lattice of subunits is balanced by lateral interactions. However, stability can be modulated by a variety of factors, including associated proteins and a variety of drug molecules. Stabilising drugs such as Taxol and the assembly-promoting repeat motifs of tau protein occupy a special pocket in β-tubulin. Microtubule destabilising drugs such as colchicine alter the longitudinal interfaces of the subunits so that they cannot assemble into a microtubule lattice. These mechanisms are discussed in terms of the atomic structure of the protein.

Introduction

The central role of microtubules in the process of separating duplicated chromosomes before cell division makes them an important target for anticancer drugs. The microtubules that make up the mitotic spindle are in a particularly delicate state of balance between assembly and disassembling into their constituent subunits. This is because both the formation of the spindle and the movement of the two sets of chromosomes to opposite spindle poles depends on carefully coordinated extension and shrinkage at the ends of the microtubules in the spindle. Thus, to understand exactly how antimitotic drugs like Taxol, discodermolide, the epothilones, colchicine or vinblastine work and possibly to design better ones, we need to understand in detail the mechanisms that control microtubule assembly and disassembly.

The subunit lattice of microtubules

Unpolymerised tubulin exists as a tight αβ-tubulin heterodimer with two bound molecules of guanosine triphosphate (GTP). A microtubule is a cylinder of linear polymers (protofilaments) in which the tubulin heterodimers are arranged head to tail in a polar fashion. It can be seen by electron microscopy (EM) that each protofilament consists of globular 4 nm subunits (Fig. 1a); however, α- and β-tubulin are so similar in structure that the two kinds of monomer subunits cannot be distinguished by EM, except at very high resolution. Monomers in adjacent protofilaments are slightly staggered (Fig. 2) so they also form a set of shallow helices, which make a complete turn over an axial distance of 12 nm. The 2D lattice of tubulin monomers is best revealed in diffraction patterns derived from electron micrographs of microtubules (Fig. 1b).

In vivo, the microtubule cylinders usually have 13 protofilaments, though the number may be different in particular situations. *In vitro*,

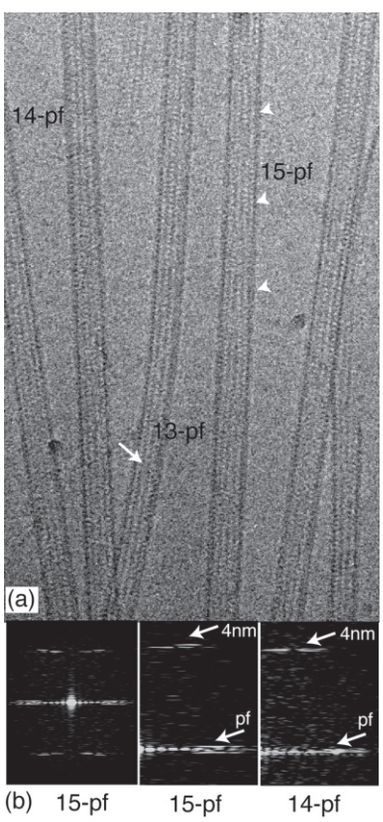

Fig. 1 (a) Electron microscopy of microtubules assembled *in vitro*. The microtubules shown were assembled from pure pig brain tubulin and rapidly frozen in a very thin layer of ice. Since frozen hydrated specimens are unstained, all the contrast comes from the difference between protein and ice. Microtubules with different numbers of protofilaments (pf) vary in diameter. Also, because their protofilaments run at slightly different angles to the microtubule axis, the Moiré patterns created by superposition of the front and back layers of the tubes have different appearances. Protofilaments in a 13-pf segment of a microtubule run straight (white arrow). White arrowheads indicate repeats in the Moiré pattern of a 15-pf microtubule. (Image kindly provided by J. Fan). **(b) Diffraction patterns** were obtained by calculating the 2D Fourier transforms of individual microtubule images and displaying their amplitudes. For well-ordered specimens, the patterns show a 'layerline' of peaks at a reciprocal height above the origin of 1/40 Å, arising from the 40 Å longitudinal spacing of the tubulin monomers. Along the equatorial line, there are peaks arising from the ~50 Å lateral separation of the protofilaments (pf). The precise pattern of peaks provides information about how much the tubulin lattice is rotated and, hence, about the number of protofilaments.

I studied physics as an undergraduate in Oxford but began working on the structures of biological particles when Aaron Klug in Cambridge gave me the chance to help develop computer programmes to reconstruct 3D images of spherical viruses and various helically symmetrical filaments from electron micrographs. I became particularly fascinated with microtubules and have used electron microscopy to study their structure and interaction with other proteins, including those that control microtubule assembly and the motor proteins that move along them.

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it is possible for purified tubulin to assemble with a fairly wide range of diameters and to contain 9–18 protofilaments (Figs. 1a and 2). This variation reveals that there is some flexibility in the bonds between adjacent protofilaments, at least in the direction involved in curvature of the microtubule wall. However, the 2D lattice of subunits needs to be rotated slightly, in one or other direction, in order to close up neatly into tubes with larger or smaller than standard diameters (Fig. 2). When there are 13 protofilaments, they run straight allowing microtubule-associated motor proteins, such as dynein and kinesin,[1,2] to run for long distances along their microtubule tracks without switching lanes; the transport of vesicles and mitochondria along axons, for example, would be more problematical if they needed to rotate around microtubules. If microtubules have more or fewer than 13 protofilaments and local interactions between subunits in the lattice are preserved, the lattice rotates and the protofilaments wind with a long pitch around the microtubule axis.[3,4] *In vivo*, however, the occurrence of microtubules with protofilaments numbers other than 13 appears to be determined by isoforms with specific changes in the amino acid sequences of α- and β-tubulin, as for example in the case of some specialised 15-protofilament microtubules in neurons of the nematode *Caenorhabditis elegans*.[5,6] These changes may allow the formation of a slightly deformed lattice in which a larger number of protofilaments can still be straight.

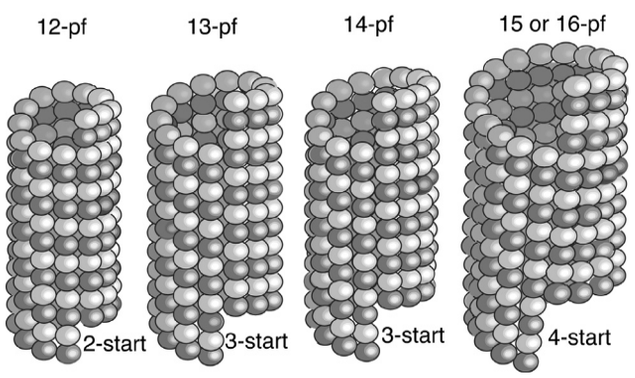

Fig. 2 Microtubules with varying numbers (12–16) of longitudinal protofilaments (pf). When 13 protofilaments make up the cylinder, they run straight, but larger or smaller numbers must wind slowly around the axis if the monomer subunits are to line up correctly at the seam. The tilting of the subunit lattice is most obvious in the 15- or 16-pf structure. In this lattice, a line through laterally adjacent monomers runs along a shallow helix. For 13- or 14-pf tubes, three shallow helices run in parallel and form a '3-start' set. Narrower or wider tubes have 2 or 4 shallow helices. In this drawing, monomer subunits are represented as darker and lighter spheres to distinguish between α- and β-tubulin. The lattice shown, known as the B-lattice, is one in which all or most lateral interactions are between like monomer subunits. Perfect helical symmetry (with all lateral interactions alike) is possible for B-lattice microtubules with some pf numbers, such as 12, 15 or 16, but a standard 13-pf microtubule can only close with a 'seam' where each α-tubulin monomer makes lateral contact with a β-tubulin subunit. The so-called A-lattice is one in which all lateral contacts would be like this.[7] Most microtubules assembled *in vitro* from pure tubulin have a B-lattice of 12–14 pfs with an A-lattice-like seam. Because of their instability, the lattices of native microtubules are difficult to investigate.

Dynamic instability

The end of a microtubule that terminates with β-tubulin is more dynamic than the other end, which has an α-tubulin monomer as its final subunit. In cells, microtubules usually grow out from some sort of organising centre (see Fig. 3a) from which the more dynamic end, known as the plus end, is able to grow and shrink, while the minus end may not be able to change. If both ends are free, as *in vitro*, assembly and disassembly can occur from either end, though at different rates. Microtubules can continue to grow as long as the free tubulin concentration is above a critical level. The critical concentration at a minus end is somewhat higher than at a plus end and thus minus ends tend to stop growing first.

Even when the tubulin concentration is above the critical level, however, it is observed that any microtubule end may suddenly stop growing and begin to shrink rapidly. The switch is a stochastic process; individual plus ends shrink rapidly while others are still growing.[10] The change from growth to shrinkage has been termed a 'catastrophe'. After a while, a shrinking microtubule end may 'pause' and/or begin to grow again; the latter process is known as a 'rescue'. Microtubules tend to disassemble when cells are cooled below their normal temperature and reassemble when they are rewarmed but they show dynamic instability even under constant warm conditions.

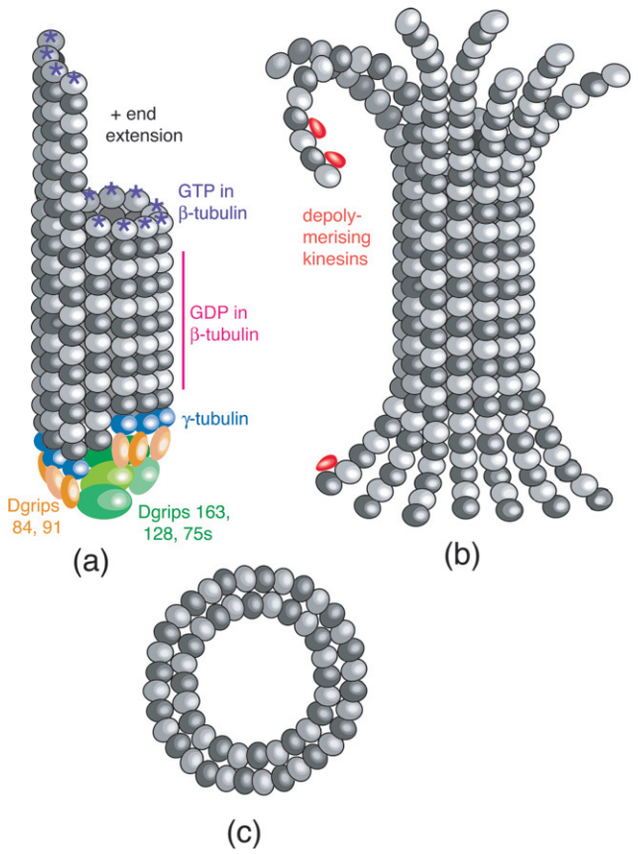

Fig. 3 Growing and shrinking microtubule ends and protofilament rings. (a) Shows a microtubule growing at its plus end. A narrow sheet of protofilaments often grows ahead of the rest of the tube. The minus end of this microtubule is shown capped and stabilised by a γ-tubulin ring complex.[8] γ-Tubulin is coloured blue and Dgrips proteins are represented as green and orange ovoids; (b) shows a microtubule that is shrinking at both ends. This can happen spontaneously. However, the cell can control depolymerisation using specialised kinesin motors, from the KinI family (shown in red).[9] After depolymerisation, protofilaments may form a variety of spirals as well as single or double-ring (c) structures.

GTP hydrolysis

The hydrolysis rate of GTP by unpolymerized tubulin dimer is very low (0.054 min^{-1} at most[11,12] but it dramatically increases during the polymerization into microtubules 21 min^{-1}).[13] The GTP bound to α-tubulin is non-exchangeable, being trapped between the 2 monomers of the heterodimer (the so-called N-site), and is apparently never hydrolysed. However, when heterodimers associate to form protofilaments, GTP on β-tubulin is hydrolysed to guanosine diphosphate (GDP) as a consequence of the interaction with α-tubulin in the next dimer. This interaction will be discussed in more detail later. It is not clear how soon this exchangeable GTP (on the so-called E-site) is hydrolysed after the addition of a new dimer to the plus end but it may be very quick. The 'cap' of dimers containing GTP on a microtubule end may be as little as one subunit deep.[14] Unpolymerised tubulin dimer with GDP bound has a curved conformation. However, the GDP microtubule structure that exists throughout most of the microtubule is constrained to form straight protofilaments by contacts between neighbouring subunits in the lattice, which has been proposed to store conformational energy and to release it during depolymerization.

The GTP on an FtsZ monomer, the bacterial monomeric homologue of tubulin,[15] is apparently hydrolysed in a similar way during

the assembly of FtsZ protofilaments, although there is some evidence that hydrolysis may be delayed in this case.[16] If FtsZ protofilaments assemble *in vitro* without any lateral support, they may disassemble immediately after GTP has been hydrolysed. Although FtsZ assembles into protofilaments very like those formed by tubulin,[17,18] it does not associate in the same way into microtubules; indeed, the form of the the polymer that is active *in vivo* is as yet unknown. All that is clear is that a ring of filaments is assembled at the centre of a dividing cell,[19] close to the membrane, and that the contraction of this ring is necessary for cell division. The role of FtsZ in prokaryotes is thus more akin to that of the contractile ring of actin filaments in animal cells than to the role of microtubules in eukaryotic chromosome separation.

Assembly of microtubules appears to take place mainly through addition of individual heterodimers to the ends of protofilaments. During rapid assembly at the plus end, there is usually a group of protofilaments in part of the microtubule wall that takes the lead,[20] so that a narrow sheet may extend for some distance beyond the end of the fully closed tube (Fig. 3a). Such sheets have not been seen at minus ends, where growth is more even. Disassembly at either end appears to be a cooperative process, since the ends of disassembling microtubules have been seen splaying apart and bending outwards into a curved conformation (Fig. 3b). In some circumstances, long segments of protofilaments are shed as spirals and 30–40 nm diameter rings can form (Fig. 3c). It has been postulated that catastrophes, pauses and rescues at the plus end are caused by random loss or restoration of the GTP tubulin cap. However, microtubules assembled *in vitro* also display dynamic instability at their minus ends, where the content of GTP is not thought to vary. Thus, the stochastic events responsible for changes in behaviour may be spontaneous conformational changes that are propagated along either individual protofilaments or small groups of protofilaments. The bending of protofilaments during disassembly is a visible manifestation of one such cooperative change.

In microtubules, nucleotide hydrolysis produces a small conformational change that shows up as a 2–4% reduction in the length of the tubulin dimer. This was discovered by comparing microtubules assembled with GTP with those assembled with GMPCPP (guanylyl-(α,β)-methylene-diphosphonate).[21] While the microtubules hydrolyse GTP quickly and thus have GDP bound to most of their β-subunits, they hydrolyse GMPCPP very slowly and the microtubules can be seen in a GTP-like state. The difference in longitudinal spacing can be measured in diffraction patterns (Fig. 1b). Microtubules assembled with GMPCPP are relatively stable. The nucleotide-dependent change in spacing is indicative of a conformational change that puts the protofilaments into a strained, but still straight, condition responsible for the dynamic instability of microtubules.

As already mentioned, the unconstrained GDP state is curved. A surprising observation is that when a protofilament forms a ring (Fig. 3c), it appears to bend at all the interfaces between monomers, both between and within heterodimers,[22] even though there is still GTP in the intradimer interface. FtsZ protofilaments also form rings when the monomers contain GDP.[23,24] On top of the observed variations in the numbers of protofilaments in microtubules assembled *in vitro*, and hence variations in their curvature, whole microtubules can bend and twist without snapping or coming under great elastic strain. This is apparent from images of fluorescently labelled microtubules growing and shrinking in living cells.[25] When a microtubule bends, individual protofilaments are bent in a variety of directions. It is likely, therefore, that there are multiple 'bent' states for dimers and protofilaments. Thus there is no guarantee that the curved conformations induced by different agents of disassembly (see below) should be identical.

γ-tubulin complexes

The protein complexes that make up microtubule-organizing centres usually include a third kind of tubulin known as γ-tubulin, which probably does not hydrolyse GTP, although there is tentative evidence that it may be capable of assembling into protofilaments as well as making lateral contacts.[26,27] The complexes present at microtubule minus ends also include various special microtubule-associated proteins (MAPs), known as Dgrips in the case of the *Drosophila* complexes that have been studied in detail.[8,28] γ-Tubulin complexes have been detected in the form of ~25 nm diameter rings in centrosomes and have been proposed to provide a template for the 13-protofilament microtubule lattice.[8]

Fig. 3a shows the model in which the rings act as basal templates for 13-protofilament microtubules. An alternative model, based on the conservation of both of the interfaces involved in the formation of protofilaments, depicts the γ-tubulin ring as a rolled-up, stored form of a γ-tubulin protofilament that can straighten to stabilize an αβ-tubulin microtubule lattice by lateral interactions.[29]

Atomic structure

The atomic structure of tubulin in one assembled conformation is known from a 3.5 Å resolution map obtained by electron crystallography of zinc-induced polymers.[30,31] As shown in Fig. 4, the overall fold of the polypeptide is closely related to that of FtsZ.[32] Each protein has a pair of globular domains set on either side of a central (core) helix (H7). The larger globular domain, comprising the N-terminal half of the polypeptide, has the same fold as a family of dinucleotide binding proteins with the so-called Rossmann fold.[15,33] There is a binding site for the guanosine nucleotide on the plus end surface of this domain, where contact is made with the "activation" domain of the next subunit in the protofilament. The position of the nucleotide at the centre of the polymerization interface prevents its exchange from subunits embedded in the microtubule. The activation domain of β-tubulin has a binding site for Taxol, which also makes contact with the core helix, on the opposite side from its contact with the nucleotide base. The C-terminal end of each tubulin polypeptide forms two long helices (H11 and H12) connected by a U-turn (see also Fig. 5) while the final 13 residues of α-tubulin and final 9 residues of β-tubulin are too disordered in the 2D crystals to show up as electron density but are assumed to project out into the solution. The C-terminal region of FtsZ is quite different in structure (Fig. 4).

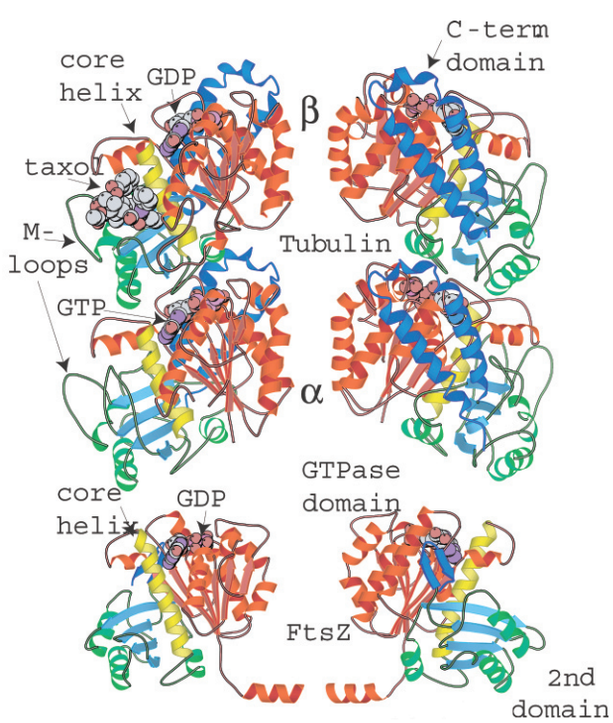

Fig. 4 Ribbon diagrams of tubulin dimer and FtsZ monomer structures. The tubulin dimer is from bovine brain,[30] the FtsZ from *Methanococcus jannaschii*.[32] On the left are views corresponding to the inside view of a microtubule while on the right are outside views. α-Helices are coloured orange in the GTPase domain and green in the second globular domain, which can now be called the 'activation domain' (J. Löwe, personal communication). β-Sheet strands are pink and light blue in these two domains, respectively. The core helix that connects the two domains is coloured yellow. The C-terminal domain is coloured blue. (prepared using Molscript.[34])

The other main differences from FtsZ are found in the loops connecting different α-helices and β-strands, those in tubulin being significantly longer. Some of these are involved in lateral contacts between the protofilaments in a microtubule. A number of workers have docked protofilaments from the zinc-sheet structure into lower-resolution helical microtubule maps,[35–38] There is general agreement that the 'M-loops' of one protofilament make contact with the GTPase domains of the next one, in the region between helix H3 and the β-sheet (Fig. 5). In zinc-induced sheets, they make different contacts with the adjacent protofilament, on the surface that corresponds to the outside of a microtubule. This provides a reason why kinesin is unable to bind to tubulin in these sheets. The 8 Å resolution microtubule map of[37] shows that the M-loops are also in a slightly different conformation in a microtubule compared with a zinc-induced sheet.

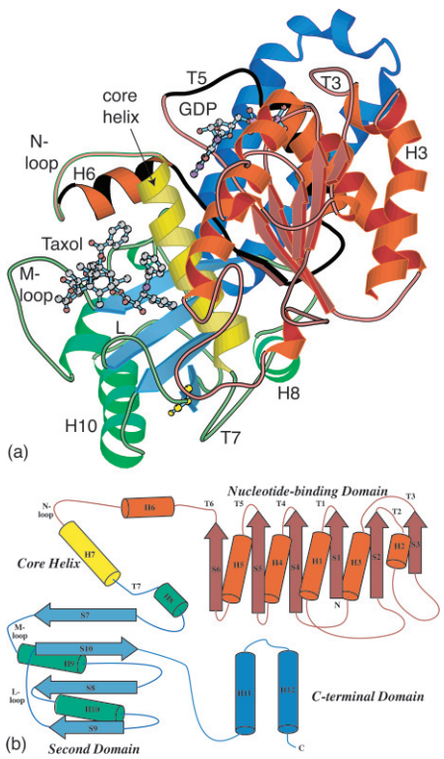

Fig. 5 Atomic model of β-tubulin and its topology scheme.The upper panel shows a ribbon diagram similar to that in Fig. 4a, with the same colour scheme, while the lower panel demonstrates how the different structural elements are connected in the primary structure.[15] Taxol is represented as a ball and stick model sitting in the pocket on the top left, above the S9–S10 loop (L). There is evidence that colchicine binds in the region of Cys354,[39] shown as a yellow ball-and-stick model, near to the surface that binds to α-tubulin in the heterodimer. Vinblastine binds within the region of residues 177–215 (T5–H6, traced in black);[40] most probably the site is close to the interface that binds to α-tubulin in another heterodimer. (prepared using Molscript.[34])

Loops T1 to T6 of the GTPase domains and T7 of the activation domains make direct contact with the nucleotides, which sit between adjacent monomers in a protofilament (see Figs 4 and 5). These loops are regions of high sequence homology between tubulin and FtsZ. The same interactions seen in tubulin protofilaments are thought to occur in FtsZ protofilaments.[18] The high affinity of the site for nucleotide has been demonstrated by a remarkable experiment with FtsZ.[41] Unlike tubulin, which is very unstable, the FtsZ of *Methanococcus jannaschii* could be refolded after being unfolded with guanidinium hydrochloride. Nucleotide was released during unfolding of the protein but as much as 80% rebound when the denaturant was diluted 50-fold in fresh buffer and the FtsZ refolded.

The mechanism of GTPase activation in tubulin and FtsZ differs from the mechanism of classical GTPase activating proteins. During assembly, the T7 loop and helix H8 are brought close to the phosphates of the nucleotide in the active site of the next subunit, indicating that this region of tubulin or FtsZ acts as its own hydrolysis-activating protein.[15,42] This has been confirmed experimentally by mutagenesis of FtsZ.[43–45] There are several totally conserved residues in loop T7 and others at the lower end of helix H8 in α-tubulin and FtsZ. In particular, FtsZ has an aspartic acid residue and α-tubulin a glutamic acid residue in H8, close to the γ-phosphate position. Mutation of the Asp to Ala in the FtsZ of *Escherichia coli* completely abolishes GTP hydrolysis, without significantly affecting nucleotide binding.[43] This residue and the equivalents in tubulin (α:Glu254 and β:Lys254) are fully conserved in all known FtsZ and tubulin sequences, respectively. If FtsZ:Asp238 and α:Glu254 are essential for hydrolysis, it seems likely that the reason GTP bound to α-tubulin is never hydrolysed is that β-tubulin has lysine in place of glutamic acid on its contact surface.

The binding site and conformation of Taxol bound to β-tubulin

With data to 3.5 Å resolution, at best (even this is a very remarkable achievement given that the data were obtained from very tiny 2D crystals, using electron microscopy and electron diffraction), atomic resolution was modelled by docking amino acid residues with standard stereochemistry into the electron density.[30] The model was refined[31] to make the modelled electron density agree as far as possible with the experimental data but the limited resolution means that the positions of individual atoms are still fairly uncertain. The uncertainty is greatest in the direction 90° to the plane of the sheets because of the impossibility of collecting data when the EM support grid is tilted at a large angle relative to the electron beam (leading to a 'missing cone' of data).

Taxol inhibits chromosome transport in a dividing cell because it binds to tubulin and permanently stabilises microtubules. It does this by sitting in a pocket in β-tubulin above the β-sheet of the second domain and next to the core helix (Figs 4 and 5). This space corresponds to the extended S9–S10 loop in α-tubulin. Interestingly, FtsZ has an empty pocket in the equivalent position. This may indicate the existence of stabilising components in bacteria and may be a potential binding site for antibiotic drugs to inhibit bacterial division. Fortunately, the structure of Taxol has been solved to high resolution repeatedly, by X-ray crystallography and NMR. Snyder *et al.*[46] have correlated the electron density in the Taxol-binding pocket in the tubulin map with all the known Taxol conformations and have identified the one which is most likely to be present. This is a T-shaped or butterfly-like structure, opened-up to expose a hydrophobic surface that interacts with a hydrophobic patch on the surface of β-tubulin. Its shape is similar to the extra portion of the S9–S10 loop in α-tubulin and presumably has a similar effect on tubulin monomer stability. The question of what might normally occupy the pocket in β-tubulin is discussed below.

Other stabilising drugs

New microtubule-stabilizing compounds that have promise for cancer treatment include the epothilones,[47,48] discodermolide,[49] eleutherobin[50,51] and the sarcodictins.[51] Despite the apparent structural diversity of these compounds,[52] all seem to compete with Taxol for binding to microtubules, apparently because they bind, with a higher affinity than Taxol, to the same pocket on β-tubulin. Electron crystallographic studies also indicate that Taxol, discodermolide and the epothilones have overlapping binding sites (K. H. Downing, personal communication). Comparative structural information about how these different drugs bind to tubulin will be very useful in illuminating the important features of the interactions that stabilise the straight conformation.

Assembly inhibiting agents

A variety of drugs are known to inhibit microtubule assembly,[53] and thereby stall cells in mitosis, when microtubules are most dynamic and least stable. It seems likely that most of these compounds, if not all of them, favour the curved conformation of tubulin. The best-known drug, colchicine, is thought to bind to β-tubulin near to the interface between dimers in a protofilament (Fig. 5). Bai *et al.*[39] found evidence, from crosslinking of colchicine analogues binding

to 2 cysteine residues in β-tubulin, Cys-239 and Cys-354, and deduced by modelling that a likely binding site was located at the α/β interface but that a significant conformational change must occur in the transition to the unpolymerized state to allow colchicine to fit in the binding site. Photo-cross-linking of colchicine to tubulin[54,55] had earlier indicated a similar binding site. The location is also in agreement with results from fluorescence energy transfer experiments that put the colchicine and Taxol-binding sites 17 Å apart, whereas the distance between colchicine and the exchangeable nucleotide is outside the range of the technique.[56] A colchicine-binding site at the interface between monomers fits a mechanism in which binding of the drug causes a distortion of the dimer structure that inhibits its polymerization into straight protofilaments and thence into microtubules. Such a distortion has recently been observed in a crystal structure of tubulin bound to colchicine.[57] However, to explain why binding of colchicine also stimulates GTP hydrolysis, it seems necessary to suppose that colchicine-bound tubulin dimers can go through a cycle in which they form transient complexes that break up again after hydrolysis of GTP.

Vinblastine can turn protofilaments into fairly tightly wound helices. Like colchicine, it binds to β-tubulin according to a cross-linking experiment that identified a binding region somewhere on residues 175–213.[40] This peptide includes regions that are involved in longitudinal polymerization contacts between dimers (Fig. 5). A water-soluble synthetic compound, cemadotin,[58] and members of the cryptophycin family all seem to suppress microtubule dynamics by reducing the rates of shrinkage as well as growth. *In vitro* studies of cryptophycin-52 have shown that only five or six molecules of drug per microtubule are needed to halve microtubule dynamics. This suggests that a cap of drug-bound tubulin subunits can stabilize the microtubule ends.[59] Unfortunately, cryptophycins have apparently been rejected as anticancer drugs after undergoing clinical trials but the compounds remain useful in structural investigations. Cryptophycin-1 induces the formation of 240 Å diameter rings, containing eight tubulin dimers,[60] significantly smaller than the rings that form naturally (12–16 dimers, average bend per monomer 15°–11°, see Fig. 3c). Watts *et al*.[60] obtained 26Å resolution EM images that distinguished intradimer contacts from interdimer contacts and showed 13° and 32° bends in these two positions, respectively. The drug was also found to protect both α- and β-tubulin against proteolysis by trypsin, indicating conformational changes in specific regions of both subunits. They concluded that drug binding to the β-subunit affected both subunits in some way.

Conformational changes at atomic level

The straight state of tubulin solved by Nogales and colleagues[31] corresponds to the shorter straight conformation and is, presumably, closer to the "strained" state than to the initial GTP-bound state, even though it was stabilised with Taxol. The change in subunit length upon GTP hydrolysis most probably involves movement of some of the loops around the nucleotide that are also involved in longitudinal bonds. However, the core helix (connected at one end to loop T7 and at the other end to the H6–H7 loop (N) that contributes to both longitudinal and lateral inter-subunit contacts) also may shift, tilt or even shorten by melting at one or other of its ends. The core helix provides a possible means of communication from the top to the bottom of the β-subunit and a change in its conformation might explain how hydrolysis of the nucleotide bound to β-tubulin could lead to changes in α-tubulin as well.

A curved, and presumably unstrained, dimer conformation has been seen in cocrystals of tubulin and the protein stathmin (op18), with and without colchicine bound to the tubulin.[57,61] Each stathmin molecule interacts laterally with a pair of tubulin heterodimers, while its amino-terminal domain caps one of the β-tubulin subunits, preventing its interaction with any other tubulin dimers. The resolution of 3.5 Å achieved in the second paper showed interesting differences from the straight tubulin conformation. In agreement with the EM studies of the curved state, there was a ~12° bend between the α- and β-tubulin subunits in a heterodimer as well as a similar bend between dimers; bending at both interfaces was observed even in the absence of colchicine. Within each subunit, a relative rotation was observed between the activation domain and the GTPase domain, to which the C-terminal helices appeared to be rigidly tethered. The rotation was measured as 8° in α-tubulin and 11° in β-tubulin. Finally, contacts between subunits in the curved protofilament structure were preserved by local movements of helices H6, H7 and H8 and loop T5.

Since the FtsZ structure was solved by X-ray diffraction of protein crystallized in a disassembled state,[32] it was proposed[15] that some of its conformational differences from the straight tubulin structure might correspond to differences between the polymerized and non-polymerised states. If two FtsZ molecules are juxtaposed with the T7 loop of one FtsZ monomer in contact with the nucleotide on the next monomer, as would be expected in a protofilament, there are several clashes that may indicate a conformational change in the molecule upon polymerization.[15,18] When the FtsZ and straight tubulin structures are compared (Fig. 6), with the GTPase domains superimposed optimally, there is a small shift in the core helix H7 and the whole activation domain is rotated. The positions of H7 and H8 in the FtsZ structure are similar to those in the curved tubulin structure but helices H9 and H10 and the beta sheet of the activation domain have moved in a different direction. Several new crystal forms of FtsZ proteins, including one with an empty nucleotide binding site, now show the same conformation as the original crystals (J. Löwe, personal communication). It is hoped that, before long, some crystals will contain FtsZ in the assembled straight form and show whether a rotation between the GTPase and activation domains actually occurs in this protein. Similarly, a crystal structure of tubulin in the unpolymerised state is needed. The recently published stathmin–tubulin structure[57] is still in an assembled state, albeit a curved one. It remains to be seen what are the conformations of either the soluble tubulin dimer, with the nucleotide binding site of β-tubulin accessible for exchange, or the GTP-filled assembled structure with a longer dimension than the assembled ADP-containing tubulin dimer.

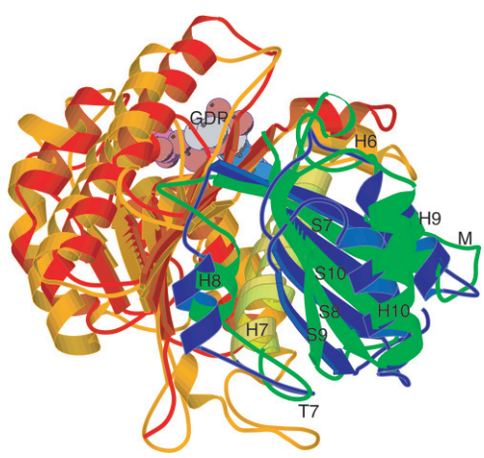

Fig. 6 FtsZ superposed on β-tubulin. The GTPase domain of tubulin is coloured orange, that of FtsZ red. Both core helices (H7) are yellow. The activation domain of tubulin is green, that of FtsZ blue. The core helices, loops T7 and the β-sheets of the GTPase domain are in good alignment. The α-helices of the GTPase domain are also reasonably well aligned. However, the activation domains need to be rotated to make them overlap.(superimposed using MSDfold (http://www.ebi.ac.uk/msd-srv/ssm/) and drawn using Molscript.[34])

Stabilising effects of MAPs

In nature, Taxol and other microtubule-stabilising drugs are found only in specific groups of organisms. Yet they bind to a site in tubulin that is very highly conserved. It is likely, therefore, that these poisons are taking the place of some natural stabilising agents found in most cells. Their great diversity suggests that none of the drugs may closely resemble the natural substrate. The microtubule-associated proteins (MAPs) are a possible set of candidates for this role. One very widespread group of MAPs, which includes the mam-

malian neuronal proteins MAP2 and tau, has a microtubule binding domain containing several copies of a conserved motif (Fig. 7). These repeat motifs are found in homologous proteins that have been isolated from a wide range of species.[62,63]

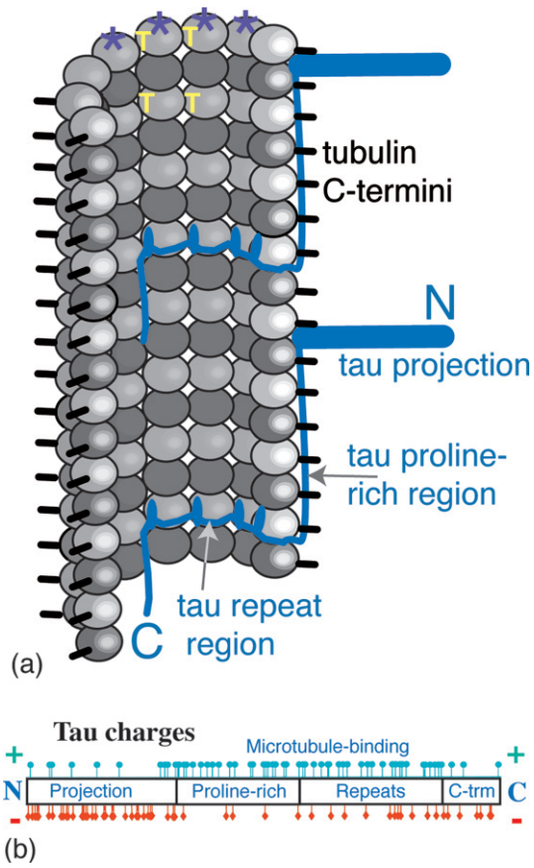

Fig. 7 Model of the interaction of mammalian tau molecules with a microtubule.(a) Diagram showing part of a microtubule with the inside surface exposed. The very acidic C-terminal segment of each tubulin monomer is exposed on the outer surface (small black projections). Tau molecules are shown in blue. T = Taxol in β-tubulin, * = GTP in the β-tubulin 'cap'.(b) Arrangement of the various domains of human 4-repeat tau, showing the distribution of basic and acidic amino acid residues. (modified from[64])The fairly acidic N-terminal segment of tau forms a projection (shown in (a)), that is repelled by the negatively charged tubulin surface. The proline-rich region, with a net positive charge, is thought to interact quite strongly with the microtubule surface. The repeat region has a net positive charge but much less so than the proline-rich region. It consists of 3 or 4 semi-conserved repeat sequences containing motifs similar to the extended loop in α-tubulin.[65] These motifs each bind to β-tubulin in the pocket that corresponds to the extended loop in α-tubulin *i.e.* where Taxol has been seen to bind.

There is now evidence that at least part of the motif binds to a site on β-tubulin that overlaps with the Taxol-binding site.[65] This was shown by labelling one of the repeat motifs of tau with a nanogold particle and localising the gold by 3D analysis of electron micrographs. The specimens were microtubules assembled with tubulin and tau and then decorated with kinesin motor domains. The latter bind stoichiometrically, one motor domain per tubulin heterodimer, and the shape of this complex is sufficiently asymmetric to define which of the two tubulin subunits is α and which is β. Difference maps between microtubules containing labelled and unlabelled tau gave a small peak on the inside surface of β-tubulin. Supporting evidence that Taxol and discodermolide both compete with tau for overlapping binding sites came from binding assays in which pelleted microtubules contained less tau in the presence of these drugs.[65,66] Furthermore, part of the tau repeat motif has the sequence THVPGGN, resembling the conserved sequence TVVPGGDL in the extended loop of α-tubulin.

Regions of tau outside the repeat region are thought to bind to sites on the outer surface of microtubules and to support a domain that projects out from the surface. The projection domain is thought to have important roles in determining the spacing between microtubules in axons and possibly in binding to other structures such as the axonal membrane. A model of the arrangement of a complete tau molecule in a microtubule is shown in Fig. 7. Clearly this arrangement depends on co-assembly of tubulin and tau, which would happen naturally *in vivo*. Other models in which tau binds only to the outer surface[67] have been derived from experiments in which preassembled microtubules were stabilised with Taxol before the addition of tau.

Conclusion

Mechanisms of stabilisation and destabilisation

Microtubule-stabilising drugs such as Taxol and the assembly-promoting repeat motifs of MAPs like tau protein all seem to occupy a pocket in β-tubulin that corresponds to an extended loop in α-tubulin. These structures are in contact with the core helix and with the M-loop. One proposed mechanism for stabilising the assembled state is that structures occupying these pockets stabilise lateral contacts by holding the important M-loop in place.[35,37] An alternative possibility is that these structures hold the ATPase and second domains in a relative orientation that favours the straight protofilament conformation, which would otherwise be altered by GTP hydrolysis.[68] The two mechanisms may be combined.

MAPs provide two additional forms of stabilisation. Firstly, the loops that occupy the pockets on the inside surfaces of β-tubulin are interconnected in the repeat domain, thus crosslinking three or four dimers, probably in adjacent protofilaments.[65] Secondly, the molecules have other domains that almost certainly bind well to the outer surface of a microtubule and probably run along a protofilament covering several tubulin dimers.[66] MAPs are known to have a stiffening effect on microtubules.[69,70] Overall, their binding should both favour the straight heterodimer conformation and hold the protofilaments together.

The effect of the GTP analogue GMPCPP,[71] which tubulin can hydrolyse only very slowly, shows that the GTP-bound state of β-tubulin makes microtubules very stable. The conformation of α-tubulin is permanently stable because of the non-exchangeable, non-hydrolysed GTP as well as the presence of the extended loop. It is not really clear why it is advantageous to have a stable α-subunit, but it may be because it makes the properties of the two ends of a microtubule more distinct. GTP binds on the other side of the core helix from Taxol and the contact may exert a similar influence on the relative orientations of the GTPase domain, the core helix and the second domain. The presence of the γ-phosphate must also have a direct effect on the interface between α- and β-tubulin, strengthening the bond between heterodimers. It is notable that Taxol stabilized microtubules are relatively flexible,[69,72] suggesting that the interdimer bonds are not strengthened in this case.

The mechanism of microtubule destabilisation also seems to involve alteration of the longitudinal interfaces, in this case resulting in a distorted protofilament structure that cannot support microtubule polymerization. Disruption may be due to a drug that binds between the two monomers in a heterodimer, as in the case of colchicine, or to one like vinblastine that appears to interfere with the interaction between different heterodimers. Also, as mentioned above, bending occurs at both the intradimer and interdimer interfaces when tubulin protofilaments assume the curved state, whether in the presence or absence of drugs. Thus, destabilisation, as well as stabilisation, appears to involve communication between the two ends of the β-tubulin monomers, between the GTP-binding site and the region including T7 and H8. The core helix seems to provide the most likely means of transmitting information between these two surfaces. This cooperative mechanism cannot have evolved in order to allow these drugs to work but is presumably advantageous during the rapid disassembly phase of microtubule dynamics. It remains to be seen how communication along the protofilament axis is actually achieved. It is hoped that, before long, some really high resolution tubulin crystal structures will throw light on this important question.

Meanwhile, the development of improved microtubule-specific drugs will remain largely empirical.

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References

1 N. Hirokawa, Kinesin and dynein superfamily proteins and the mechanism of organelle transport, *Science*, 1998, **279**, 519–526.
2 R. D. Vale, The molecular motor toolbox for intracellular transport, *Cell*, 2003, **112**, 467–480.
3 D. Chrétien and R. H. Wade, New data on the microtubule surface lattice, *Biol. Cell*, 1991, **71**, 161–174.
4 R. H. Wade, D. Chretien and D. Job, Characterization of microtubule protofilament numbers – How does the surface lattice accommodate, *J. Mol. Biol.*, 1990, **212**, 775–786.
5 T. Fukushige, Z. K. Siddiqui, M. Chou, J. G. Culotti, C. B. Gogonea, S. S. Siddiqui and M. Hamelin, MEC-12, an alpha-tubulin required for touch sensitivity in *C. elegans*, *J. Cell Sci.*, 1999, **112**, 395–403.
6 C. Savage, M. Hamelin, J. G. Culotti, A. Coulson, D. G. Albertson and M. Chalfie, Mec-7 is a beta-tubulin gene required for the production of 15-protofilament microtubules in *Caenorhabditis elegans*, *Genes Dev.*, 1989, **3**, 870–881.
7 L. Amos and A. Klug, Arrangement of subunits in flagellar microtubules, *J. Cell Sci.*, 1974, **14**, 523–49.
8 M. Moritz and D. A. Agard, γ-Tubulin complexes and microtubule nucleation, *Curr. Opin. Struct. Biol.*, 2001, **11**, 174–181.
9 G. C. Rogers, S. L. Rogers, T. A. Schwimmer, S. C. Ems-McClung, C. E. Walczak, R. D. Vale, J. M. Scholey and D. J. Sharp, Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase, *Nature*, 2004, **427**, 364–370.
10 A. Desai and T. J. Mitchison, Microtubule polymerization dynamics, *Ann. Rev. Cell Dev. Biol.*, 1997, **13**, 83–117.
11 T. David-Pfeuty, C. Simon and D. Pantaloni, Effect of antimitotic drugs on tubulin GTPase activity and self-assembly, *J. Biol. Chem.*, 1979, **254**, 11696–11702.
12 H. P. Erickson, FtsZ, a prokaryotic homolog of tubulin?, *Cell*, 1995, **80**, 367–370.
13 R. Melki, S. Fievez and M.-F. Carlier, Continuous monitoring of Pi release following nucleotide hydrolysis in actin or tubulin assembly using 2-amino-6-mercapto-7-methylpurine ribonucleoside and purine-nucleoside phosphorylase as an enzyme-linked assay, *Biochemistry*, 1996, **35**, 12038–12045.
14 R. Walker, N. Pryer and E. Salmon, Dilution of individual microtubules observed in real time in vitro: evidence that cap size is small and independent of elongation rate, *J. Cell Biol.*, 1991, **114**, 73–81.
15 E. Nogales, K. H. Downing, L. A. Amos and J. Löwe, Tubulin and FtsZ form a distinct family of GTPases, *Nature Struct. Biol.*, 1998, **5**, 451–458.
16 L. Romberg and T. J. Mitchison, Rate-limiting guanosine 5′-triphosphate hydrolysis during nucleotide turnover by FtsZ, a prokaryotic tubulin homologue involved in bacterial cell division, *Biochemistry*, 2004, **43**, 282–288.
17 H. P. Erickson, D. W. Taylor, K. A. Taylor and D. Bramhill, Bacterial cell division protein FtsZ assembles into protofilament sheets and minirings, structural homologs of tubulin polymers, *Proc. Natl. Acad. Sci., U.S.A.*, 1996, **93**, 519–523.
18 J. Löwe and L. A. Amos, Tubulin-like protofilaments in Ca^{2+}-induced FtsZ sheets, *EMBO J.*, 1999, **18**, 2364–2371.
19 E. F. Bi and J. Lutkenhaus, FtsZ ring structure associated with division in *Escherichia coli*, *Nature*, 1991, **354**, 161–164.
20 D. Chrétien, S. D. Fuller and E. Karsenti, Structure of growing microtubule ends: two-dimensional sheets close into tubes at variable rates, *J. Cell Biol.*, 1995, **129**, 1311–1328.
21 A. A. Hyman, D. Chrétien, I. Arnal and R. H. Wade, Structural changes accompanying GTP hydrolysis in microtubules: information from a slowly hydrolyzable analogue guanylyl-(alpha,beta)-methylene-diphosphonate, *J. Cell Biol.*, 1995, **128**, 117–125.
22 E. Nogales, H.-W. Wang and H. Niederstrasser, Tubulin rings: which way do they curve?, *Curr. Opin. Struct. Biol.*, 2003, **13**, 256–261.
23 H. P. Erickson and D. Stoffler, Protofilaments and rings, two conformations of the tubulin family conserved from bacterial FtsZ to α/β and γ tubulin, *J. Cell Biol.*, 1996, **135**, 5–8.
24 C. L. Lu, M. Reedy and H. P. Erickson, Straight and curved conformations of FtsZ are regulated by GTP hydrolysis, *J. Bacteriol.*, 2000, **182**, 164–170.
25 V. Rodionov, E. Nadezhdina and G. Borisy, Centrosomal control of microtubule dynamics, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 115–120.
26 Y. F. Inclan and E. Nogales, Structural models for the self-assembly and microtubule interactions of gamma-, delta- and epsilon-tubulin, *J. Cell Sci.*, 2001, **114**, 413–422.
27 R. Llanos, V. Chevrier, M. Ronjat, P. Meurer-Grob, P. Martinez, R. Frank, M. Bornens, R. H. Wade and J. Wehland, et al., Tubulin binding sites on gamma-tubulin: Identification and molecular characterization, *Biochemistry*, 1999, **38**, 15712–15720.
28 D. Job, O. Valiron and B. Oakley, Microtubule nucleation, *Curr. Opin. Cell Biol.*, 2003, **15**, 111–117.
29 H. P. Erickson, Gamma-tubulin nucleation: template or protofilament?, *Nature Cell Biol.*, 2000, **2**, E93–E96.
30 E. Nogales, S. Wolf and K. H. Downing, Structure of the αβ tubulin dimer by electron crystallography, *Nature*, 1998, **391**, 199–203.
31 J. Löwe, H. Li, K. H. Downing and E. Nogales, Refined structure of αβ tubulin at 3.5Å resolution, *J. Mol. Biol.*, 2001, **313**, 1045–1057.
32 J. Löwe and L. A. Amos, Crystal structure of the bacterial cell-division protein FtsZ, *Nature*, 1998, **391**, 203–206.
33 M. G. Rossmann, D. Moras and K. W. Olsen, Chemical and biological evolution of nucleotide-binding protein, *Nature*, 1974, **250**, 194–199.
34 P. J. Kraulis, Molscript – a program to produce both detailed and schematic plots of protein structures, *J. Appl. Crystallogr.*, 1991, **24**, 946–950.
35 E. Nogales, M. Whittaker, R. A. Milligan and K. H. Downing, High resolution model of the microtubule, *Cell*, 1999, **96**, 79–88.
36 L. A. Amos, Focusing-in on microtubules, *Curr. Op. Struct. Biol.*, 2000, **10**, 236–241.
37 H. Li, D. DeRosier, W. Nicholson, E. Nogales and K. Downing, Microtubule structure at 8 Å resolution, *Structure*, 2002, **10**, 1317–1328.
38 P. Meurer-Grob, J. Kasparian and R. H. Wade, Microtubule structure at improved resolution, *Biochemistry*, 2001, **40**, 8000–8008.
39 R. Bai, D. G. Covell, X.-F. Pei, J. B. Ewelli, N. Y. Nguyeni, A. Brossi and E. Hamel, Mapping the binding site of colchicinoids on β-tubulin, *J. Biol. Chem.*, 2000, **275**, 40443–40452.
40 S. S. Rai and J. Wolff, Localization of the vinblastine-binding site on beta-tubulin, *J. Biol. Chem.*, 1996, **271**, 14707–14711.
41 J. M. Andreu, M. A. Oliva and O. Monasterio, Reversible unfolding of FtsZ cell division proteins from archaea and bacteria: Comparison with eukaryotic tubulin folding and assembly, *J. Biol. Chem.*, 2002, **277**, 43262–43270.
42 H. P. Erickson, Atomic structures of tubulin and FtsZ, *Trends Cell Biol.*, 1998, **8**, 133–137.
43 K. Dai, A. Mukherjee, Y. Xu and J. Lutkenhaus, Mutations in FtsZ that confer resistance to SulA affect the interaction of FtsZ with GTP, *J. Bacteriol.*, 1994, **175**, 130–136.
44 A. Mukherjee, C. Saez and J. Lutkenhaus, Assembly of an FtsZ mutant deficient in GTPase activity has implications for FtsZ assembly and the role of the Zring in cell division, *J. Bacteriol.*, 2001, **183**, 190–197.
45 D. J. Scheffers, J. G. de Wit, T. den Blaauwen and A. J. Driessen, GTP hydrolysis of cell division protein FtsZ: evidence that the active site is formed by the association of monomers, *Biochemistry*, 2002, **41**, 521–529.
46 J. P. Snyder, J. H. Nettles, B. Cornett, K. H. Downing and E. Nogales, The binding conformation of Taxol in beta-tubulin: a model based on electron crystallographic density, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 5312–5316.
47 D. M. Bollag, P. A. McQueney, J. Zhu, O. Hensens, L. Koupal, J. Liesch, M. Goetz, E. Lazarides and C. M. Woods, Epothilones, a new class of microtubule-stabilizing agents with a taxol-like mechanism of action, *Cancer Res.*, 1995, **55**, 2325–2333.
48 P. Giannakakou, R. Gussio, E. Nogales, K. H. Downing, D. Zaharevitz, B. Bollbuck, G. Poy, D. Sackett and K. C. Nicolaou, et al., A common pharmacophore for epothilone and taxanes: Molecular basis for drug resistance conferred by tubulin mutations in human cancer cells, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 2904–2909.
49 E. ter Haar, R. J. Kowalski, E. Hamel, C. M. Lin, R. E. Longley, S. P. Gunasekera, H. S. Rosenkranz and B. W. Day, Discodermolide, a cytotoxic marine agent that stabilizes microtubules more potently than taxol, *Biochemistry*, 1996, **35**, 243–50.
50 B. H. Long, J. M. Carboni, A. J. Wasserman, L. A. Cornell, A. M. Casazza, P. R. Jensen, T. Lindel, W. Fenical and C. R. Fairchild, Eleutherobin, a novel cytotoxic agent that induces tubulin polymerization, is similar to paclitaxel (Taxol(R)), *Cancer Res.*, 1998, **58**, 1111–1115.
51 E. Hamel, D. L. Sackett, D. Vourloumis and K. C. Nicolaou, The coral-derived natural products eleutherobin and sarcodictyins A and B: effects on the assembly of purified tubulin with and without microtubule-associated proteins and binding at the polymer taxoid site, *Biochemistry*, 1999, **38**, 3490–3498.
52 K.-H. Altmann, Microtubule-stabilizing agents: a growing class of important anticancer drugs, *Curr. Op. Chem. Biol.*, 2001, **5**, 424–431.
53 P. M. Checchi, J. H. Nettles, J. Zhou, J. P. Snyder and H. C. Joshi, Microtubule-interacting drugs for cancer treatment, *Trends Pharmacol. Sci.*, 2003, **24**, 361–365.

54 R. Bai, X. F. Pei, O. Boye, Z. Getahun, S. Grover, J. Bekisz, N. Y. Nguyen, A. Brossi and E. Hamel, Identification of cysteine 354 of beta-tubulin as part of the binding site for the A ring of colchicine, *J. Biol. Chem.*, 1996, **271**, 12639–12645.
55 S. Uppuluri, L. Knipling, D. L. Sackett and J. Wolff, Localization of the colchicine-binding site of tubulin, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 11598–11602.
56 Y. Han, H. Malak, A. G. Chaudhary, M. D. Chordia, D. G. Kingston and S. Bane, Distances between the paclitaxel, colchicine, and exchangeable GTP binding sites on tubulin, *Biochemistry*, 1998, **37**, 6636–6644.
57 R. B. G. Ravelli, B. Gigant, P. A. Curmi, I. Jourdain, S. Lachkar, A. Sobel and M. Knossow, Insight into tubulin regulation from a complex with colchicine and a stathmin-like domain, *Nature*, 2004, **428**, 198–202.
58 M. A. Jordan, D. Walker, M. de Arruda, T. Barlozzari and D. Panda, Suppression of microtubule dynamics by binding of cemadotin to tubulin: possible mechanism for its antitumor action, *Biochemistry*, 1998, **37**, 17571–17578.
59 D. Panda, K. DeLuca, D. Williams, M. A. Jordan and L. Wilson, Antiproliferative mechanism of action of cryptophycin-52: Kinetic stabilization of microtubule dynamics by high-affinity binding to microtubule ends, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 9313–9318.
60 N. R. Watts, N. Cheng, W. West, A. C. Steven and D. L. Sackett, The cryptophycin-tubulin ring structure indicates two points of curvature in the tubulin dimer, *Biochemistry*, 2002, **41**, 12662–12669.
61 B. Gigant, P. A. Curmi, C. Martin-Barbey, E. Charbaut, S. Lachkar, L. Lebeau, S. Siavoshian, A. Sobel and M. Knossow, The 4A X-Ray structure of a tubulin:stathmin-like domain complex, *Cell*, 2000, **102**, 809–816.
62 M. Goedert, C. P. Baur, J. Ahringer, R. Jakes, M. Hasegawa, M. G. Spillantini, M. J. Smith and F. Hill, PTL-1, a microtubule-associated protein with tau-like repeats from the nematode *Caenorhabditis elegans*, *J. Cell Sci.*, 1996, **109**, 2661–2572.
63 G. Heidary and M. E. Fortini, Identification and characterization of the *Drosophila tau* homolog, *Mech. Dev.*, 2001, **108**, 171–178.
64 M. Goedert, R. Jakes, M. G. Spillantini and R. A. Crowther, (1994). Tau protein and Alzheimer's disease. In *Microtubules*. (Hyams, J. and Lloyd, C., Eds.), Wiley, New York.
65 S. Kar, G. J. Florence, P. I. and L. A. Amos, Discodermolide interferes with the binding of tau protein to microtubules, *FEBS Lett.*, 2003, **539**, 34–36.
66 S. Kar, J. Fan, M. J. Smith, M. Goedert and L. A. Amos, Repeat motifs of tau bind to the insides of microtubules in the absence of taxol, *EMBO J.*, 2003, **22**, 1–8.
67 J. Al-Bassam, R. S. Ozer, D. Safer, S. Halpain and R. A. Milligan, MAP2 and tau bind longitudinally along the outer ridges of microtubule protofilaments, *J. Cell Biol.*, 2002, **157**, 1187–1196.
68 L. A. Amos and J. Löwe, How taxol stabilises microtubule structure, *Chem. Biol.*, 1999, **6**, R65–R69.
69 R. B. Dye, S. P. Fink and R. C. J. Williams, Taxol-induced flexibility of microtubules and its reversal by MAP2 and tau, *J. Biol. Chem.*, 1993, **268**, 6847–6850.
70 H. Felgner, R. Frank, J. Biernat, E.-M. Mandelkow, E. Mandelkow, B. Ludin, A. Matus and M. Schliwa, Domains of neuronal microtubule-associated proteins and flexural rigidity of microtubules, *J. Cell Biol.*, 1997, **138**, 1067–1075.
71 A. A. Hyman, S. Salser, D. N. Drechsel, N. Unwin and T. J. Mitchison, Role of GTP hydrolysis in microtubule dynamics: information from a slowly hydrolyzable analogue, GMPCPP, *Mol. Biol. Cell*, 1992, **3**, 1155–1167.
72 P. Venier, A. C. Maggs, M. F. Carlier and D. Pantaloni, Analysis of microtubule rigidity using hydrodynamic flow and thermal fluctuations, *J. Biol. Chem.*, 1994, **269**, 13353–60.