Focusing-in on microtubules

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A good approximation of the atomic structure of a microtubule has been derived from docking the high-resolution structure of tubulin, solved by electron crystallography, into lower resolution maps of whole microtubules. Some structural interactions with other molecules, including nucleotides, drugs, motor proteins and microtubule-associated proteins, can now be predicted.

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Abbreviations

EM	electron microscopy
GMP·CPP	guanylyl-(α,β)-methylenediphosphonate
IRR	inter-repeat region
MAP	MT-associated protein
MT	microtubule
PF	protofilament

Introduction

Understanding the precise role of microtubules (MTs) as elements of the infrastructure of the cytoplasm and their involvement in intracellular transport requires knowledge of their molecular structure at atomic level. The assembly of MTs from αβ-tubulin heterodimers (see Figure 1) and their dynamic behaviour [1] are controlled by a variety of factors, including an exchangeable nucleotide bound to β-tubulin and specific MT-associated proteins (MAPs) that lower the critical concentration for assembly. Motor proteins carry vesicular and other cargo along their surfaces. This review considers what recent new structural information reveals about these interactions.

Conformation of assembled tubulin

The 2D sheets used in determining the atomic structure of tubulin [2] contained longitudinal protofilaments (PFs) equivalent to those in MTs and therefore revealed normal longitudinal interactions. Normal lateral interactions have recently been established by fitting the PFs into electron microscopy (EM) maps of both undecorated MTs [3••] and MTs decorated with motor proteins [4•]. The position on the MT inside surface (Figure 1) of the binding site for paclitaxel (Taxol®) was unexpected in view of the rapidity with which paclitaxel binds to pre-assembled MTs; Diaz *et al.* [5••] found it took less than 1 min for paclitaxel to convert MTs with approximately 14 PFs into mainly 12-PF structures.

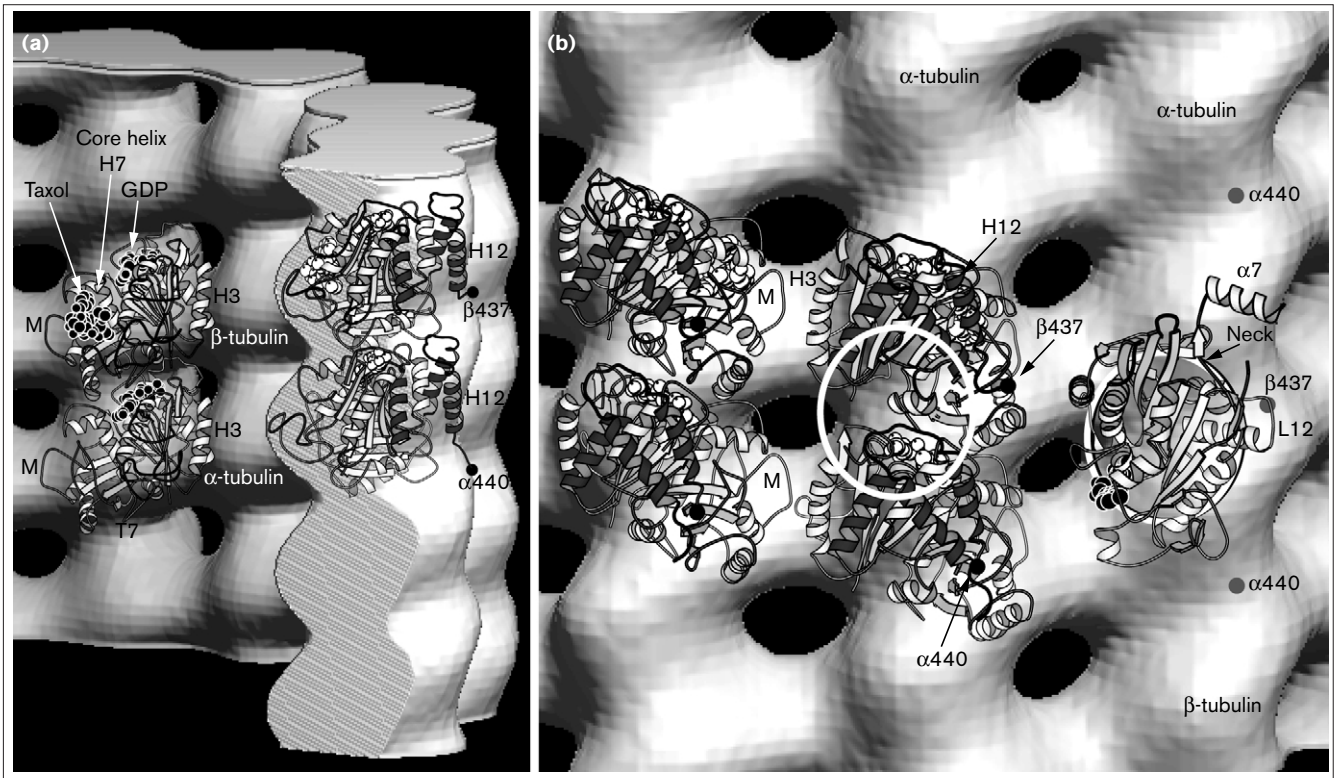
Although tubulin heterodimers assemble *in vitro* so that lateral contacts between adjacent PFs are mainly between pairs of like subunits, most of the resulting MTs also have one line of connections, the so-called 'seam', where α-tubulin monomers in one PF contact β-tubulin monomers in the next. Paclitaxel may enter the MT lumen because the 'seam' opens up periodically [5••], but it is more likely that it simply enters via gaps between PF bonds (see Figure 1). Even if individual lateral bonds are weak, a long row of them should be difficult to disrupt. Also, analysis of electron cryomicroscopy (cryo-EM) images [6] has shown that lateral bonds are stiff enough to conserve the helical lattice during large-scale deformations. This would not be true if there were slippage along a weak seam.

The *in vitro* preference for lateral associations among like subunits is reduced in some buffer conditions. Very high NaCl concentrations not only encourage assembly of 10-PF MTs, but also introduce more 'seams' [7], making MTs with mixed lateral contacts. *In vivo*, other factors, such as specific MAPs, may influence the lateral contacts and native MT lattices may vary according to the circumstances.

Nogales *et al.* [3••] established that helix H3 of the N-terminal GTPase domain of one tubulin subunit contacts the 'M-loop' of the smaller globular domain of an adjacent subunit. Paclitaxel binds to β-tubulin near the M-loop and may exert some of its stabilising effect through the lateral contacts. α-Tubulin seems to be permanently stabilised by an extended loop (S9–S10) that occupies a pocket equivalent to the paclitaxel-binding site in β-tubulin. It is intriguing that the effect of paclitaxel bound to the smaller globular domain of β-tubulin is similar to the effect of the presence of unhydrolysed nucleotide in a site at the top of the N-terminal domain. Both produce the same small increase in the spacing of subunits along tubulin PFs [5••]. GTP bound to α-tubulin is trapped in the middle of the heterodimer and is never hydrolysed; the exchangeable site on β-tubulin is exposed and GTP that binds there is hydrolysed to GDP during assembly. Hydrolysis is thought to be triggered upon the addition of a new heterodimer [8•], by contact with its T7 loop (see Figure 1) [9]. MTs normally show dynamic instability [1], but those assembled with a nonhydrolysable analogue, guanylyl-(α,β)-methylenediphosphonate (GMP·CPP), are relatively stable.

The state of the nucleotide bound to β-tubulin may be sensed by any of the surrounding loops. Nogales *et al.* [3••] suggested that loop L3 may control lateral interactions by affecting the conformation of H3, the helix that follows it. An alternative suggestion is that the core helices (H7) of tubulins and their bacterial homologue, FtsZ [9], have a controlling effect on the conformation of the whole molecule [10•]. H7 contacts both the nucleotide and either the α-tubulin extended loop or paclitaxel if it is bound to β-tubulin. This helix may act as a lever, controlling the orientations of the globular domains, as well as of loop T7 at

Figure 1



Current models of MT structure at atomic resolution. Tubulin exists as αβ-heterodimers, which are oriented in a polar fashion along the longitudinal PFs from which an MT is made. The 3D structure of tubulin, from cryo-EM data [2], represented as a ribbon model, is docked on to low-resolution 3D images of tubulin PFs in the orientation found to fit best [3••]; the MT surface structure shown is from [51]. **(a)** A section through an MT, revealing part of the inside surface; two views of the atomic structure are shown, from inside the MT (left) and from one side of a PF (right). **(b)** A view of the outside surface of an MT. PFs are oriented with their plus ends towards the top of the picture. Outside views of the tubulin atomic structure are docked on to two PFs (left and centre), the atomic structure of kinesin [52] is superimposed on the third PF (right). The subunit at the top of each tubulin heterodimer is β-tubulin, with α-tubulin closer to the MT minus end. 'M' labels the 'M-loops', which contact helix H3 in the adjacent PF. At the top of each monomer is a guanine-nucleotide-binding site; GTP (bound to α-tubulin) and GDP (bound to β-tubulin), shown as space-filling models, occupy sites in the interfaces between subunits. Here, each nucleotide is contacted by loop T7 of the next subunit in the PF. Paclitaxel, also shown as a space-filling molecule, lies in a pocket on the inside surface of the β-subunit (a). The tubulin core helix (H7) lies between the GTPase domain and the smaller globular paclitaxel-binding domain. At the tubulin C termini, the positions of the last visible residues (α440 and β437) are indicated by dark circles; the conformations of the last 8–10 residues are unknown, but H12 of β-tubulin may extend for a further nine residues under favourable conditions [53•]. White circles in (b) indicate positions on the PFs where kinesin and ncd can bind. The kinesin monomer is shown in the orientation in which it docked best into a cryo-EM map of the weakly binding ADP-filled state [40••]. In this orientation, loop L12, important in sensing the presence of MTs [16••], is positioned close to the C terminus of β-tubulin. Loops L8 and L11 are on the far surface of kinesin, facing helices H11 and H12 of β-tubulin. Kinesin's 'neck', including coiled-coil helix α7, which is essential for processive movement [16••,56••], lies in the same region. Alternative proposed dockings [42,55•,56••] are similar except for a 40–60° rotation about a longitudinal axis, putting L12 in direct contact with the PF surface.

the base of the helix. The substoichiometric effect of paclitaxel suggests there is cooperativity among tubulin subunits. Possibly, the line of H7 helices linking successive nucleotide-binding sites via T7 loops (Figure 1) forms a backbone along the PF, so the effect of tilting one core helix may be transmitted to successive 'vertebrae'.

A study with an MT-depolymerising motor [11••] has revealed an interesting difference between the effects of paclitaxel and GMP·CPP; when MTs are assembled with paclitaxel, the products of disassembly are tubulin dimers, whereas in the presence of GMP·CPP, PFs are not dissociated, but roll up into spirals. This suggests that the effect that the state of the nucleotide has on the interdimer contact is not necessarily coupled to conformational changes associated with the bending/straightening of the PFs. When normal kinesin or ncd bind strongly to tubulin, the β-monomer appears tilted compared with its position in undecorated MTs [4•,12]; destabilising motors such as Kar3 [13] and Kin I kinesins [11••] may have a similar, but more pronounced, action.

Studies of 2D sheets of FtsZ PFs [14•] affirm their close homology to tubulin PFs, except that they consist of

monomers not heterodimers. The hydrolysis of GTP at the top of an FtsZ monomer appears to be controlled by contact with the T7 loop at the bottom of the next monomer. FtsZ PFs even appear to have associated proteins with some homology to MAPs [15•].

Microtubule-associated proteins

The definition of MT-associated proteins (MAPs) as 'proteins that attach to MTs *in vivo*' [16••] encompasses a steadily growing and very diverse group (e.g. [17,18,19•,20,21•–23•,24–26,27•–29•]). Members of a more narrowly defined family, 'proteins that bind (*in vitro*) in a nucleotide-insensitive manner to the MT lattice' [1], contain a variety of repeated MT-binding motifs [30••,31,32•–34•]. The 'classical' model for their activity is that each motif binds to a separate tubulin subunit and that much of the stabilising effect of such MAPs results from cross-linking adjacent tubulin dimers [1]. But this does not explain how these MAPs, at very low molar stoichiometric levels relative to tubulin, suppress MT dynamics [1] and even affect MT rigidity [35]. It is likely that MAPs also affect the conformation of tubulin PFs in analogous ways to paclitaxel and GMP·CPP.

Recent work [30••] illustrates the complexity of the interaction between the MAP tau and tubulin. The adult form of tau has a MT-binding C-terminal domain that includes four 18-residue repeat motifs, separated by 13–14-residue inter-repeat regions (IRRs). Juvenile forms of tau (also found in adults) have only three repeat motifs. These three or four repeat motifs are known to stabilise MTs, whereas a separate proline-rich region binds strongly to tubulin, but does not by itself promote assembly. Within the four-repeat region, it has previously been shown [36] that one of the IRRs (the 10 residues of R1–R2) possesses more than twice the binding affinity of a repeat motif and is, by itself, sufficient to promote tubulin polymerisation. The authors [36] suggest that the role of this IRR is to increase the stability of adult MTs by anchoring tau to the tubulin lattice, whereas three-repeat tau molecules that lack it, predominant in immature neurons, are more mobile to allow developmental plasticity.

Having the proline-rich region, as well as the repeat region of tau enhances MT assembly *in vitro* by 10-fold [30••]. This cooperative effect suggests that a tau molecule, which has no detectable secondary structure in solution, folds up so that distant parts of the molecule specify the way that the stabilising motifs interact with tubulin. Nonconsecutive segments of tau may even interact with the same tubulin subunit. *In vitro*, it is apparently possible to saturate MTs with one tau molecule to every two tubulin dimers [33•,36]; it may be that only the most strongly binding repeat motifs are then occupied.

The R1–R2 IRR can be cross-linked to the approximately 12-residue acidic C-terminal segments of both α- and β-tubulin, whereas a subset of other repeat motifs binds to more internal sites in the C-terminal thirds of α- and β-tubulin [30••]. If these sites are close to the M-loops, the repeat motifs may have a role in controlling lateral bonds. The presence of neuronal MAPs causes tubulin PFs in zinc-induced sheets to move approximately 2 Å further apart [37], presumably involving a movement of the M-loops.

MAP2 and MAP4 both have C-terminal domains similar in structure and activity to those of the tau family [32•,33•], but it has been reported that only 50% of a saturating level of MAP2 (one molecule per tubulin dimer) on MTs was released by competition with tau [33•]. The subtle cooperation between stabilising and cross-linking motifs of MAPs means that even closely related molecules can have different properties and dissimilar effects on dynamic instability parameters, such as rates of growth and shrinkage, and rates of catastrophe and rescue [16••], and on treadmilling [38•].

Motor-protein-binding sites

There seems to be some overlap between binding sites for MAPs and motor proteins [39] in the region of the tubulin C termini (Figure 1). *In vivo*, transfection of tau into cells decreases the run length of vesicles moving along MTs [34•].

Kinesins

3D cryo-EM has shown that motor domains of the kinesin family bind to tubulin across both subunits of a heterodimer (Figure 1) [12,40••], covering part of the surface of the GTPase domain of β-tubulin, as well as part of the ridge that contains the C-terminal domains (helices H11 and H12) of both tubulins [4•]. It is not yet clear which point on this surface is mimicked by a naturally occurring kinesin inhibitor [41•].

Docking kinesin's atomic coordinates into EM maps of MT–motor protein complexes has led to a range of solutions [42], but a recent model [40••] has the advantage that kinesin's stalk would not clash with the MT surface. It also suggests that the head–head interaction seen in kinesin dimer crystals [43] is not merely a crystal artefact. At the same time, it is consistent with MT-dependent protection of kinesin from proteolysis [44]. An important prediction is that the kinesin L12 loop, implicated in MT stimulation of ATPase activity [16••,45••], only makes a close contact with tubulin when the motor domain is strongly bound, being held away from the surface of β-tubulin at the weakly bound ADP-filled stage of the hydrolysis cycle (Figure 1).

Although it has only a single head, the kinesin KIF1A is able to move along a MT for a distance of a micron or more without detaching [45••]. Compared with normal (dimeric) kinesin, members of the KIF1A subfamily have an extended L12 loop, containing six extra lysine residues. The exceptionally high affinity of KIF1A for MTs is probably due to the presence of this extended loop [45••], stopping the motor from diffusing away between power strokes. It is likely that loop L12 tethers KIF1A to one or both of the acidic C termini of the tubulin heterodimer (see Figure 1). Similarly, a run

of lysines along one side of helix α7 in the neck of dimeric kinesin may contribute to kinesin's processive activity.

Dyneins

Although cytoplasmic dyneins have a pair of heads that may cooperate in motility [46], a single-headed species of axonemal dynein has also been shown to be processive; single motor molecules are even sufficiently well tethered that they can be pulled backwards without detaching [47••]. Helices H12 of both α- and β-tubulin [48] and their C termini [49•] are implicated in dynein motility. Both axonemal and cytoplasmic dyneins bind in an ATPase-sensitive manner to MTs via a loop on the end of an antiparallel coiled-coil stalk [50].

Conclusions

The stability of tubulin polymers is determined by the conformations of the subunits in the lattice. GTP or GMP·CPP directly strengthen intra-PF bonds, whereas paclitaxel probably stabilises lateral bonds via the M-loop. Both nucleotide and paclitaxel contact the core helix of β-tubulin and may thus promote the straight PF conformation. The action of MAPs is complex because of multiple MT-binding motifs; they probably have a cross-linking, as well as a conformational, effect. Although their productive interactions probably occur in different places on the MT surface, both MAPs and motor proteins also make use of the acidic C termini of tubulin, which are probably important for anchoring MAPs in place and for tethering motors between active steps. These interactions will be better understood when structural biologists succeed in crystallising tubulin complexed with domains of the associated proteins.

Note added in proof

Kinesins

Amongst numerous interesting papers that have appeared very recently, one by Okada and Hirokawa [54••] strengthens the case for an interaction between the glutamate-rich C termini of tubulin and the extended, lysine-rich L12 loop of KIF1A (see Figure 1). The removal of either tubulin's C-terminal glutamates by subtilisin digestion or the six lysines in L12 of KIF1A by mutagenesis eliminated the tethering of KIF1A to MTs. This interaction between glutamates and lysines, which stops KIF1A from losing contact with the MT between ATP-driven steps, is only effective in the weakly bound ADP-filled state. In the strongly bound states, it has been proposed that kinesin's L12 loop and the adjacent helix α4 move closer to the MT surface, where they may contribute to the strong interaction [4•]. A reconstructed image [55•] of KIF1A bound to MTs in a strongly bound state clearly shows the extended L12 loop as a protrusion from the motor domain making contact with the ordered part of the tubulin surface. The identity of the protrusion was confirmed by labelling L12 with a gold cluster. In a similar way, Rice *et al.* [56••] labelled residues within both the catalytic domain of conventional kinesin and the 'neck linker' downstream of the catalytic domain. These two new structural studies [55•,56••] limit the possible range of ways (see Figure 2 of [57]) that kinesin and related motors dock on to the surface of a MT. It is now almost certain that kinesin's interaction with MTs involves loops L11 and L8, as well as L12. The recent studies of kinesin [56••,58] also reveal the crucial importance of the neck for motility.

Cytoplasmic dynein

Koonce and Tikhonenko [59••] have made a significant advance towards understanding dynein-MT interactions by performing alanine scanning of the loop at the end of the hairpin stalk. The first half of its sequence is homologous to the repetitive MT-binding region of MAP1B; the rest has no obvious homology, but both parts appear to be required for binding. Although its structure suggests a role as some sort of tether, this loop seems to be essential for any binding, weak or strong, of dynein to MTs and its attachment is sensitive to ATP.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- •• of outstanding interest

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