How Taxol® stabilises microtubule structure

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The structure of tubulin shows paclitaxel (Taxol®) binding to a pocket in β tubulin on the microtubule's inner surface, which counteracts the effects of GTP hydrolysis occurring on the other side of the monomer.

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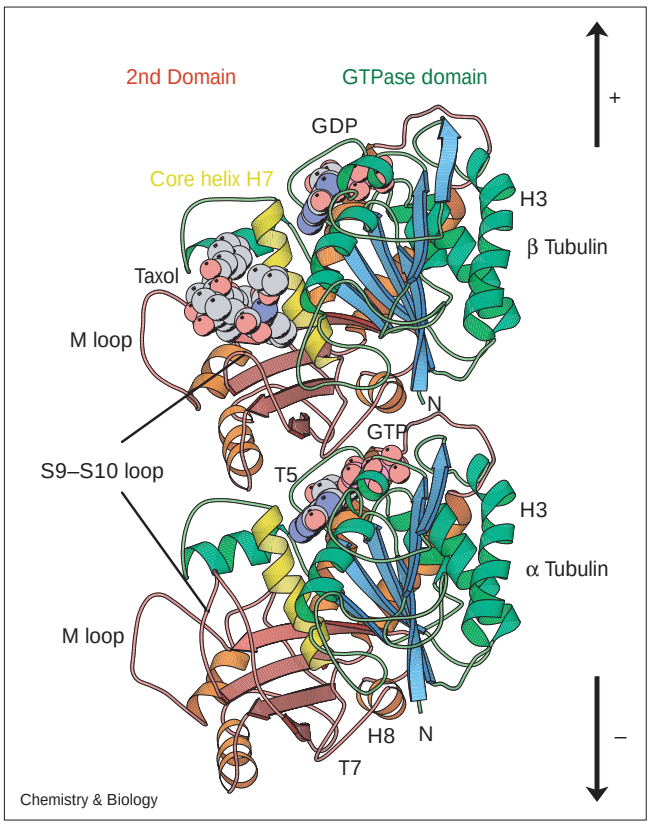
Microtubule atomic structure at last!

A year ago, a small group of electron microscopists [1] published a long-awaited model for the atomic structure of tubulin, the protein that makes up the 25 nm microtubules found in most eukaryotic cells. As a bonus, the anti-mitotic drug paclitaxel (Taxol®), an increasingly important anticancer agent, was shown attached to its binding site on the protein. The three-dimensional map of the molecular complex (Figure 1), with a resolution of 3.7 Å, was calculated from electron-microscope images and electron-diffraction patterns obtained from two-dimensional tubulin crystals suspended in thin films of amorphous ice. The interpretation of the structure at the atomic level was supported by a 2.8 Å resolution map of FtsZ protein, the bacterial homologue of tubulin, published simultaneously but solved quite independently using conventional X-ray diffraction methods [2]. In a recent paper, Nogales *et al.* [3] now show how tubulin can be fitted into microtubules and throw light on the role of paclitaxel in stabilising tubulin polymers.

Tubulin exists as α–β heterodimers oriented in a polar fashion along the longitudinal protofilaments that make up a microtubule. The two-dimensional crystals used to determine the atomic structure of tubulin also consisted of protofilaments, but an anti-parallel arrangement, induced by the presence of zinc ions, resulted in extended flat sheets rather than curved sheets that can close into tubules. The studies therefore showed not just the atomic structures of individual tubulin monomers but also how they interact to form heterodimers and how the heterodimers interact to form protofilaments. A guanine-nucleotide-binding site is found at one end of each monomer subunit, situated in a globular amino-terminal domain with a Rossmann fold. The sites on β tubulin or FtsZ are exposed when the subunits are in solution and GTP, which binds to these sites, is hydrolysed to GDP during microtubule assembly. A molecule of GTP bound to α tubulin is permanently trapped in the heterodimer by an adjoining β-tubulin monomer, however. The tubulin map shows GTP bound to α tubulin and GDP, as well as paclitaxel, bound to β tubulin. Paclitaxel binds to the second globular domain of β tubulin, on the other side of the core helix from the GTPase domain (Figure 1).

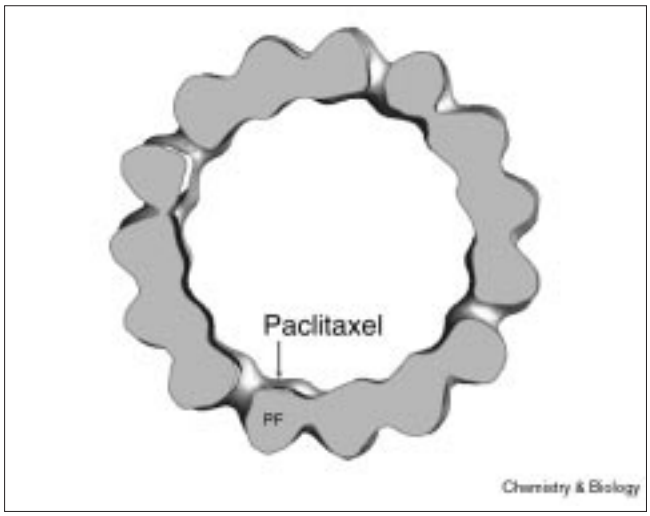
Polymers in which the β tubulin subunits contain a non-hydrolysable analogue of GTP, such as guanylyl-(α,ß)-methylenediphosphonate (GMP·CPP), are relatively stable. After hydrolysis, however, microtubules and sheets become unstable structures, because the preferred conformation for

Figure 1



The three-dimensional conformation of cow tubulin dimers, as predicted from a 3.7 Å resolution map determined using electron crystallography [1]. The atomic structures of only a handful of protein have been solved using electrons rather than X rays and this is the first time the technique has been successfully applied to a macromolecular structure other than highly crystalline sheets of membrane proteins. Arrows show the orientation relative to the more dynamic (plus) and more stable (minus) ends of a microtubule.

Figure 2



View from the minus end of a microtubule model in which the atomic coordinates of the tubulin dimer were docked by Nogales *et al.* [3]. PF, protofilament. Figure is adapted from [21].

individual protofilaments is curved rather than straight; protofilaments strain to bend outwards and disassemble in the form of rings and coils. This nucleotide-dependent conformational change is responsible for the dynamic assembly/disassembly properties of tubulin [4]. FtsZ monomers also assemble into dynamic protofilaments with similar properties [5]. If paclitaxel is bound to β tubulin, however, stable polymers form, even when the nucleotide-binding site contains GDP. This is true for the zinc-induced protofilament sheets, as well as for normal polymers.

Nogales *et al.* [3] have docked the atomic coordinates of tubulin protofilaments, including the nucleotides and paclitaxel molecule, into a three-dimensional density map calculated from electron-microscope images of microtubules, with a resolution of 2 nm (Figure 2). Even at this resolution, the protofilaments show sufficient asymmetry for the docking process to identify a unique orientation. The result agrees with other information on the orientation of the heterodimer. It was already known that β tubulin crowns the plus (dynamic) end of the microtubule, whereas the minus (more stable) end finishes with α tubulin. The molecule must therefore be oriented as shown in Figure 1.

Filling the pockets for stability

Paclitaxel binds into a pocket in the second globular domain of β tubulin, facing the central hole in a microtubule (Figure 2). The corresponding space in α tubulin is occupied by an eight-residue insertion in the loop between β strands S9 and S10 (Figure 3). This insertion is also absent from the S9–S10 loop in FtsZ [6]. The map shows paclitaxel making a close contact with the shorter S9–S10 loop in β tubulin (Figure 1), in agreement with the finding that mutations in this loop reduce the toxicity of paclitaxel to cancer cells. The molecule also appears to touch the core helix and approach the loop between S7 and H9, which Nogales *et al.* [3] have designated the 'M' loop. The taxane ring, which associates with the M loop, is not constrained by the dimer structure but becomes strongly immobilized after polymerization of tubulin [7].

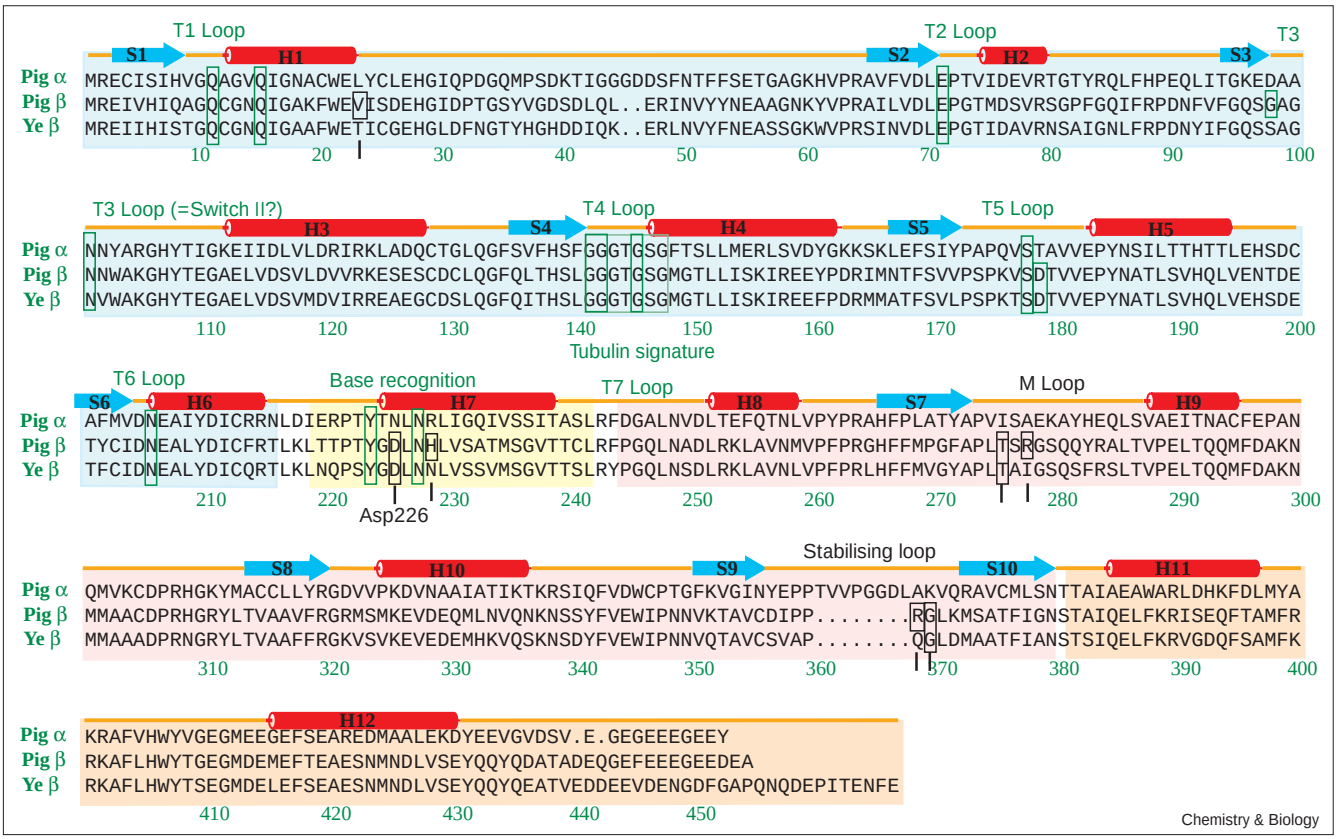
A close-up view of the binding pocket is shown in Figure 4; the amino-acid residues in closest contact with the paclitaxel molecule are labelled. There are some conserved differences in these residues between α- and β-tubulin sequences but other residues are quite variable. The precise effect of paclitaxel on microtubule dynamics depends on the assembled isotypes of β tubulin [8]. The most conserved of the β-tubulin residues in the pocket is Asp226. This residue is also present in yeast β tubulin, which is unaffected by paclitaxel, and none of the other residues directly involved in the binding pocket seems particularly unusual in yeast (Figure 3). It has to be concluded that the overall shape of the binding pocket, which is influenced by all the residues in the vicinity, is likely to determine whether paclitaxel binds there or not.

Paclitaxel is a poison produced by endophytic fungi [9] that reside in a variety of plants including the original source of the drug, the pacific yew *Taxus brevifolia*, and other members of the Taxaceae family. A growing number of other natural reagents is being discovered that seem to stabilize microtubules in the same way, including epothilones A and B from a myxobacterium *Sorangium cellulosum*, eleutherobin from a marine soft coral and discodermolide from a deep-sea sponge. Such molecules might mimic an endogenous cellular factor, presumably a less potent one, that helps to stabilise microtubules in normal cytoplasm; the natural reagent might be a small molecule or part of a microtubule-associated protein (MAP).

The M loop seems to be involved in lateral contacts between protofilaments in microtubules and in zinc-induced sheets. It makes close contacts on the adjacent protofilament with H3 and parts of the H2–S3 and H1–S2 loops. Lateral interaction surfaces are more electrostatic and less hydrophobic than the longitudinal contacts. The 'stickiness' of the M-loop side of the protofilament appears to be largely responsible for the polymorphic nature of tubulin polymers, because normal protofilament sheets may pair via two such edges to produce an S shape in cross-section [10] and this edge can even associate with regions of the outside surface of assembled microtubules and sheets to give 'hook' shapes in cross-section [11].

Whilst microtubules remain assembled, a conformational change resulting from the hydrolysis of the nucleotide to GDP is apparent as a 3–6% shortening of the ~4 nm average

Figure 3



Comparison of the sequences of mammalian α and β tubulins with β tubulin from yeast (*Saccharomyces cerevisiae*). The secondary structure of cow tubulins [1] is shown above the pig sequences. Coloured boxes indicate the three-dimensional structural domains: the GTPase domain is blue, the core helix is yellow, the second globular domain is pink and the outer-surface domain is orange. Residues that line the paclitaxel-binding pocket of mammalian β tubulin are boxed in black. Paclitaxel does not stabilise yeast β tubulin, although each residue in this pocket is the same as in one or more paclitaxel-sensitive species. Elsewhere, however, there are significant differences between yeast and mammalian tubulins. Residues directly involved in nucleotide binding are boxed in green. Note that the nucleotide base and paclitaxel contact opposite sides of the core helix.

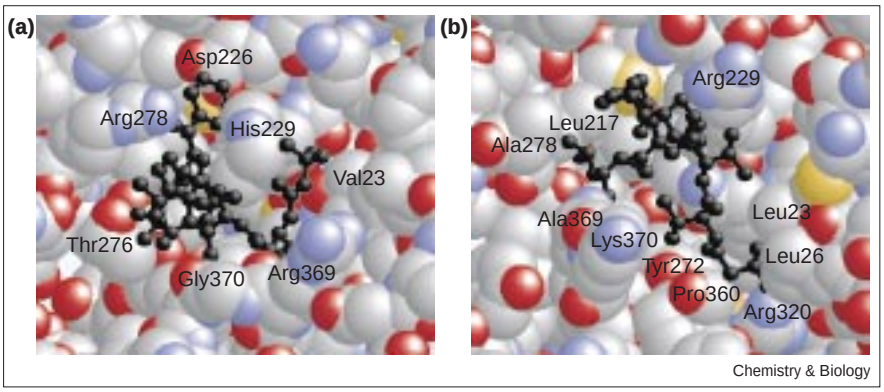
spacing of tubulin monomers [12]. The solved atomic structures provide some clues about the possible nature of this change. Although the tubulin map shows α and β tubulin to be remarkably similar, the height of GDP–β-tubulin is 2–3% less than that of GTP–α-tubulin and much of this difference is due to differing conformations of loops such as T5 and T7 (Figures 1 and 3) that make the interdimer and intradimer contacts. In addition, the FtsZ monomer structure may provide an approximate model of the disassembled β-tubulin conformation: although the core structure of FtsZ is very similar to that of both tubulin monomers, the second domain appears to be rotated by about 11°; also, the FtsZ monomer is 7.5% longer than the assembled tubulin monomers, which seems to be due mainly to a shift in the positions of helix H8 and loop T7 that are attached to the end of the core helix [6].

Changes in tubulin spacing due to assembly and GTP hydrolysis may, therefore, be explained by relatively small movements. Only small structural changes such as these are needed to greatly alter the strength of inter-subunit interactions. Nogales *et al.* [3] suggest that outwards curling of disassembling protofilaments might simply reflect a weakening of interdimer bonds at lower radii. But before disassembly, any such movement would be restrained by lateral interactions with adjacent protofilaments.

Support from different directions

The interplay between nucleotide hydrolysis and microtubule-stabilising factors that bind to the paclitaxel site is of particular interest. Microtubule and sheet stability must depend on the combined strengths of longitudinal and lateral bonds. If the interdimer longitudinal bond is weakened by the loss of γ-phosphate, paclitaxel may compensate by strengthening the lateral bonds. Paradoxically, there have been several reports that paclitaxel makes microtubules more flexible, although the reverse effect has also been seen [13–15]. Greater flexibility might imply

Figure 4



Close-up view of (a) paclitaxel bound to β tubulin and (b) the extended protein loop in α tubulin, showing that they occupy equivalent pockets on the surfaces of these subunits.

that the lateral bonds are more free to move. The main participants in normal lateral bonding are the M loop on one side and helix H3 on the other; in the zinc-induced sheets, the M loop interacts with the H11–H12 loop, and with H4 and H5. The taxane ring is in a position to influence the conformation of the all-important M loop. Its effect on the conformation of lateral contacts is demonstrated by the finding that paclitaxel reduces the average number of protofilaments in microtubules assembled from purified tubulin from 14 to 12 [16].

How can one explain the way that paclitaxel over-rides the instability due to the presence of GDP rather than GTP in β tubulin? Nogales *et al*. [3] discuss the possibility that paclitaxel-induced changes in the M loops of one protofilament could compensate for a movement in helix H3 in the neighbouring protofilament; H3 could 'sense' the loss of γ-phosphate via loop T3 after nucleotide hydrolysis. But the fact that the M loop contacts a different region on neighbouring protofilaments in zinc sheets suggests that nucleotide-dependent changes in H3 might not be crucial. It is also difficult to explain how paclitaxel would stabilise open tubulin sheets by supporting interactions with neighbouring protofilaments; there should always be an unstable protofilament on one edge and, when this disassembled, the next would become unstable.

Another puzzle is that the effects of paclitaxel appear to depend on whether it is added before or after assembly. When paclitaxel was added to pre-assembled microtubules, the only structural change was a slight shift in the relative positions of adjacent protofilaments [17], which can be explained by the effect of paclitaxel on the M loop. When microtubules were assembled in the presence of the drug, however, the longitudinal spacing of monomers was equivalent to that of GMP·CPP microtubules, suggesting that a conformational change normally associated with nucleotide hydrolysis had not occurred. It seems unlikely that lateral contacts established before nucleotide hydrolysis would be sufficient to inhibit longitudinal contraction.

The alternative explanation for the effects of taxol is that there is close communication between domains of the same subunit. Especially after assembly, loop T3 is probably more constrained than the Switch II loop of classical GTPases; forces due to the loss of the γ-phosphate from the tri-nucleotide could therefore be transmitted to the base, which contacts the core helix. The central position of this helix led us to suggest that it might act as a lever in the structure of FtsZ [2]. An inward movement of the core helix is likely to influence the orientation of the second globular domain, and the 11° rotation between the tubulin and FtsZ second domains represents the sort of conformational change one would expect. At least part of the effect of paclitaxel might be to counteract this mechanism.

Finally, an interesting recent finding is that paclitaxel is particularly effective as an anticancer agent because tubulin is not its only target. It also binds to a protein called Bcl-2 [18,19], which normally blocks the process of apoptosis, or cell death. Paclitaxel therefore plays a dual role in destroying dividing cells, firstly by stabilising assembled microtubules and thereby halting mitosis, and secondly by inhibiting Bcl-2 and allowing apoptosis to proceed. These properties have made paclitaxel an important first-line treatment for ovarian and breast cancer. Other compounds such as epothilone B appear to affect mitosis alone [20], which is likely to be useful in other circumstances.

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