The role of tubulin–tubulin lattice contacts in the mechanism of microtubule dynamic instability

Szymon W. Manka* and Carolyn A. Moores*

Microtubules (MTs) are key players in cell division, migration, and signaling and are thus targets for herbicides, fungicides, and in the treatment of many human diseases, most notably cancer. Their cylindrical lattice is built through longitudinal (head-to-tail) and lateral (side-by-side) supramolecular assembly of the tubulin α/β heterodimer. In solution, this heterodimer favors a ‘bent’ conformation, but the lattice geometry imposes and temporarily stabilizes a tense ‘straight’ conformation1–3 (Fig. 1a).

Such spring-like conformational strain lies at the heart of MT dynamic instability and is vital for MT function4,5. The crucial mechanism of microtubule dynamic instability is driven by the α-subunit’s catalytic E254 residue7 during MT polymerization. The equivalent residue within the intradimer interface is a noncata- lytic βK254, hence the N-site GTP is never hydrolyzed. E-site GTP hydrolysis somehow renders the MT wall unstable. Recent high-resolution cryo-EM structures using GTP analogs show6,10, in line with earlier studies1,4, that hydrolysis is accompanied by a longitudinal interdimer lattice compaction (~2 Å), but it is not known how this rearrangement is linked to MT catastrophe. How is the strain energy stored in the lattice released to depolymerize MTs and do work in the cell?

α- and β-tubulin subunits are structurally very similar. Each comprises three functional domains6,7 determining MT polarity: (i) a GTP-binding N-terminal (N) domain, located toward the faster-growing MT plus (+) end, (ii) an intermediate (I) domain, facing the MT minus (−) end, and (iii) a C-terminal (C) domain protruding from the MT wall (Fig. 1a). Tight ‘head-to-tail’ dimerization of α/β-tubulin buries the GTP-binding site of the αβN domain at its interface with the βI domain1, making it a nonexchangeable site (N-site), always occupied by GTP. The corresponding region in the β-subunit is exchangeable (E-site), and when occupied with GTP, it mediates analogous but metastable head-to-tail association with the αβI domain of another dimer, forming protofilaments (PFs). Individual PFs are compatible with the relaxed (energetically favored) bent tubulin conformation, but the lateral association of tubulin via specific interlocking of N and I domains gradually straightens tubulin dimers as well as the PFs formed by them1,4 (Fig. 1a). Thus, the mature MT geometry imposes and temporarily stabilizes the straight tubulin conformation1–3, and MT wall integrity relies on N- and I-domain involvement in both longitudinal and lateral lattice contacts (Fig. 1a). In vitro, MTs polymerize with a range of PF numbers, but in cells most MTs have a 13-PF architecture. This architecture necessitates that at one site, called the seam, α- and β-tubulins form heterotypic lateral contacts, breaking the otherwise helical symmetry of an MT cylinder. Thus 13-PF MTs are pseudohelical.

As described above, tubulin strain is inherent to construction of an MT wall and is something of a time bomb, awaiting the trigger for disruption. This trigger involves GTP hydrolysis at the interdimer interface: the β-subunit’s GTP is hydrolyzed due to recruitment of the α-subunit’s catalytic E254 residue1 during MT polymerization. The equivalent residue within the intradimer interface is a noncatalytic βK254, hence the N-site GTP is never hydrolyzed. E-site GTP hydrolysis somehow renders the MT wall unstable. Recent high-resolution cryo-EM structures using GTP analogs show6,10, in line with earlier studies1,4, that hydrolysis is accompanied by a longitudinal interdimer lattice compaction (~2 Å), but it is not known how this rearrangement is linked to MT catastrophe. How is the strain energy stored in the lattice released to depolymerize MTs? The vital structure of the GDP•Pi intermediate has remained unknown, although GTPγS has been thought to mimic it6,13–15.

The imbalance between subunit strain energy and lattice binding energy leading to catastrophe is predicted to result from perturbation of lattice contacts during and/or after lattice compaction. To detect these likely subtle (in the range of ~2 Å) changes in lattice contacts, it is necessary to compare high-resolution MT structures representing sequential steps in the GTPase cycle in a uniform MT architecture. This has not been achieved to date. We used double-cortin (DCX), a unique MT-associated protein (MAP) and a perfect tool for this work, as it nucleates and stabilizes a physiological 13-PF MT architecture by binding between four tubulin dimers in all nucleotide states (Fig. 1a,d) without perturbing known GTPase-linked transitions16,17. Crucially, DCX’s robust MT nucleation activity allowed rapid (~30 s) sample preparation, such that we could capture the earliest stages of DCX-MT polymerization (Fig. 1b), including the long-sought GDP•Pi transition state. By focusing on only the 13–PF MT architecture in the presence of only one MT binding partner—in contrast to previous work6,10—our structures shed new light on the structural transitions that drive dynamic instability.

Institute of Structural and Molecular Biology, Department of Biological Sciences, Birkbeck, University of London, London, UK.
* e-mail: s.manka@mail.cryst.bbk.ac.uk; c.moores@mail.cryst.bbk.ac.uk

NATURE STRUCTURAL & MOLECULAR BIOLOGY | VOL 25 | JULY 2018 | 607–615 | www.nature.com/nsmb

607
Results

Determination of 13-PF structures of DCX-MTs in different nucleotide states. To elucidate the mechanism of MT catastrophe, we analyzed conformational changes in tubulin through its GTPase cycle with single-particle cryo-EM, and assessed the impact of these changes on longitudinal and lateral lattice contacts. Overall, we determined 13-PF structures of four nucleotide states of DCX-MTs: (i) a GTP-like lattice (4.4 Å resolution) stabilized by GMPCPP, a slowly hydrolysable GTP analog; (ii) a GDP•Pi lattice (4.2 Å) and (iii) a control GTPγS lattice (4.4 Å), both of which show clear density equivalent to the γ-P of GTP, but no density for the magnesium ion; and (iv) a GDP lattice (3.8 Å; Fig. 1c, Table 1, Supplementary Figs. 1–3, and Supplementary Table 1). We calculated the lattice spacing and refined atomic models of tubulin for each reconstruction (Fig. 1c, Table 1, and Supplementary Table 1). The prehydrolysis GMPCPP lattice has an extended axial repeat (83.74 ± 0.20 Å), whereas the GDP•Pi, GTPγS, and GDP lattices are compacted (81.74 ± 0.06, 82.10 ± 0.07, and 81.90 ± 0.06 Å, respectively).
respectively). The compaction of the lattice, together with the absence of magnesium density, support the interpretation that we have captured the posthydrolysis GDP•Pi state. Even 10- to 20-dimer-long multi-PF MT precursor assemblies (DCX pre-MTs) exhibited compacted lattices (Fig. 1b), emphasizing both the speed of GTP hydrolysis and the fact that the MT wall does not need to be complete for it to occur, at least with DCX bound at interdimer vertices (Fig. 1d). The overall tubulin conformation in GTPγS-DCX-MTs is in fact more similar to that in GDP•Pi-DCX-MTs (Supplementary Fig. 3) despite the presence of γ-P/γ-S (Supplementary Fig. 2). This explains the slow MT growth observed with GTPγS and highlights the unusual properties of this nucleotide analog14.

GTP hydrolysis in β-tubulin leads to uneven compression of α-tubulin. To characterize the origin of the lattice compaction in our reconstructions, we compared the different MT states by superposition on the β-subunit, which itself does not show large conformational changes10. This comparison revealed conformational rearrangements in the nucleotide-binding βT5 loop that likely cause changes in the E-site hydrogen bond network (βN domain) in the GDP•Pi state relative to the GMPCPP state (transition 1; Fig. 2a,b and Supplementary Videos 1 and 2). The βT5 loop interfaces with the adjoining tubulin subunit via the αS9 strand of the αI domain’s central β-sheet. This β-sheet is sandwiched between two interdimer anchor points: A1, a hydrophobic contact between the C-terminal end of helix αH8 plus the αH8–S7 loop and helix

---

**Table 1 | Cryo-EM data collection, refinement, and validation statistics**

|                  | GMPCPP-DCX-MT (EMDB-3961, PDB 6EWW) | GDP•Pi-DCX-MT (EMDB-3962, PDB 6EVX) | GTPγS-DCX-MT (EMDB-3963, PDB 6EVY) | GDP-DCX-MT (EMDB-3964, PDB 6EVZ) | GDP-DCX-Taxol-MT (EMDB-3965, PDB 6EW0) |
|------------------|-------------------------------------|-------------------------------------|-----------------------------------|---------------------------------|--------------------------------------|
| **Data collection and processing** | Magnification | 35,971 | 35,971 | 35,971 | 35,971 | 35,971 |
|                  | Voltage (kV) | 300 | 300 | 300 | 300 | 300 |
|                  | Electron exposure (e-/Å²) | 25 | 25 | 25 | 25 | 25 |
|                  | Defocus range (μm) | −0.4 to −2.5 | −0.4 to −2.5 | −0.4 to −2.5 | −0.4 to −2.5 | −0.4 to −2.5 |
|                  | Pixel size (Å) | 1.39 | 1.39 | 1.39 | 1.39 | 1.39 |
|                  | Symmetry imposeda | 12-fold | 12-fold | 12-fold | 12-fold | 12-fold |
|                  | Initial particle images (no.) | 11,552 | 7,727 | 9,395 | 32,256 | 18,651 |
|                  | Final particle images (no.) | 3,244 | 6,591 | 6,037 | 30,434 | 17,626 |
|                  | Map resolution (Å) | 4.4 | 4.2 | 4.4 | 3.8 | 3.8 |
|                  | FSC thresholdb | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 |
|                  | Map resolution range (Å) | 4.1−5.8 | 4.0−5.8 | 4.0−5.8 | 3.2−4.8 | 3.2−4.8 |
| **Refinement** | Initial model used (PDB ID) | 6EVZ | 6EVZ | 6EVZ | 3JAR | 6EVZ |
|                  | Model resolution (Å) | 4.5 | 4.2 | 4.4 | 3.8 | 3.8 |
|                  | FSC threshold | 0.143 | 0.143 | 0.143 | 0.143 | 0.143 |
|                  | Model resolution range (Å) | 200−4.5 | 200−4.2 | 200−4.4 | 200−3.8 | 200−3.8 |
|                  | Map sharpening β factor (Å²) | −137 | −167 | −156 | −163 | −99 |
| **Model composition** | Nonhydrogen atoms | 40,536 | 40,896 | 40,890 | 40,866 | 41,238 |
|                  | Protein residues | 5,112 | 5,166 | 5,166 | 5,166 | 5,166 |
|                  | Ligands | 24 | 24 | 18 | 18 | 24 |
| **B factors (Å²)** | Protein | 110 | 101 | 93 | 58 | 110 |
|                  | Ligand | 143 | 86 | 82 | 64 | 103 |
| **r.m.s. deviations** | Bond lengths (Å) | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 |
|                  | Bond angles (°) | 0.71 | 0.65 | 0.72 | 0.67 | 0.76 |
| **Validation** | MolProbity score | 1.66 | 1.46 | 1.41 | 1.32 | 1.55 |
|                  | Clashscore | 5.74 | 3.73 | 3.32 | 2.58 | 4.39 |
|                  | Poor rotamers (%) | 0 | 0 | 0 | 0 | 0 |
| **Ramachandran plot** | Favored (%) | 94.98 | 95.69 | 95.26 | 95.98 | 95.79 |
|                  | Allowed (%) | 5.02 | 4.31 | 4.74 | 4.02 | 4.21 |
|                  | Disallowed (%) | 0 | 0 | 0 | 0 | 0 |

aDCX does not bind at the seam of the 13-PF MTs, thus pseudohelical symmetry was applied across the 12 PFs between which DCX binds. bGold-standard resolution estimation, according to Chen et al.35
Uneven compression of α-tubulin reinforces longitudinal lattice contacts. Transitions 1 and 2 strengthen the longitudinal interdimer interface (Fig. 2d, Supplementary Fig. 4, and Supplementary Video 3). While the core of the interface is conserved among all states, transition 1 results in additional interactions between the αN terminus and βT2 loop and between the αT7 loop and helix βH1, as well as more extended interaction twists (by a further ~1 Å; Fig. 2a and Supplementary Video 1), further narrowing the gap between the dimers.

βH11 in α1; and A2, a hydrogen bond between the K336 side chain at the C terminus of helix αH10 and the K174 backbone carbonyl in the βT5 loop (Fig. 2a–c). Thus, the conformational change in the βT5 loop leads to a ~1-Å shift of the αd domain toward the β-subunit. The αN domain, connected to the αd domain via αS6–S7 strands, undergoes a more prominent displacement (up to ~2.2 Å) toward both the MT (−) end and the lumen, together with a part of the C domain associated with it. This results in an uneven compression of the whole α-subunit, globally manifested as lattice compaction. After Pi release (transition 2), the α-subunit slightly

**Fig. 2 | GTPase-dependent structural transitions strengthen the longitudinal MT lattice contacts.** a. Backbone front view and angled close-up cut-away view comparisons of different MT nucleotide states based on superposition on the β1-subunit (underlined); colored by the degree of displacement, heteroatom, or as follows: GMPCPP, red; GDP•Pi, orange; GDP, red; Mg2+, green. Tubulin domains are outlined and their overall relative movements around A1 and A2 anchor points are indicated with straight arrows. Curved arrows indicate how these transitions influence tubulin in the context of MT lattice. b. Conformational change of the T5 loop and the likely rearrangement of local hydrogen bonds (solid black lines), shown with experimental densities (labels) colored by element (carbon, gray; nitrogen, blue; oxygen, red); nucleotides are shown as sticks; dotted ovals mark the interface expansion at each step. d. Plotted PDB ePISA (http://www.ebi.ac.uk/pdbe/pisa/) calculations of the solvent-accessible surface areas (SASA) and the dissociation energies (ΔGdiss) of the longitudinal interdimer interfaces in different nucleotide states. The error bars represent three averaged interdimer interfaces generated with parallel refinements of noncrystallographic symmetry (NCS) mates in six-dimer MT wall models (see Methods).
of strand αS9 with helix βH11 (Fig. 2c), burying ~165 Å² (~10%) more of the solvent-accessible surface area and increasing the predicted amount of energy needed to dissociate the complex by ~0.8 kcal/mol (~15%; Fig. 2d). Transition 2 further increases the interface area by ~110 Å² and the dissociation energy by ~1.1 kcal/mol due to additional interactions of the αH3–S4 loop.

Fig. 3 | GTPase-dependent structural transitions weaken the lateral MT lattice contacts. a, Backbone lumenal cut-away view of lateral contacts between two adjacent PFs in different nucleotide states, aligned and colored as in Fig. 2a. Tubulin domains are outlined and their transitions around A1 and A2 anchor points are indicated with curved arrows. b, MT (+)-end view of the α2-subunits undergoing the first transition (top), and a lumenal view of the β2-subunits undergoing the second transition; close-up views of selected connections (solid lines) compromised (dotted lines) during the transitions. Atomic models are colored as in Fig. 2a or by heteroatom and are presented with experimental densities; tables summarize the likely affected lateral bonds according to measurements performed in the models. The interacting atoms are named (in parentheses) according to PDB conventions.
Uneven compression of \(\alpha\)-tubulin weakens lateral lattice contacts. It seems paradoxical that the polymer becomes unstable while longitudinal contacts become tighter after lattice compaction. This draws attention to the lateral contacts. The N and I domains are connected in the lattice by both the longitudinal and the lateral interfaces, enabling transmission of structural information between PFs. Due to the uneven compression of the \(\alpha\)-subunits during transition 1, the lattice compaction not only tightens the interdimer spaces, but also perturbs the lateral contacts; this is because the \(\alpha\)N domains move further toward the MT (−) end and lumen than do the laterally connected \(\alpha\)I domains from the neighboring PFs (Fig. 3 and Supplementary Video 4). Thus, in our GTP state model, the conformation places the lateral contacts between the \(\alpha\)-subunits close enough together (<4 Å) to support multiple points of connectivity (for example, Gly57–Glu284, His88–His283, His88–Glu284, Glu90–Lys280, and Gln128–Gln285). After hydrolysis, two of these distances (the Gly57–Glu284 and His88–His283 pairs) increase to >4 Å as measured in our models of the GDP•Pi and GDP states. For example, \(\alpha\)Gly57–\(\alpha\)Glu284 are ∼3.8 Å apart with the \(\beta\)S3–H3’ loop and the \(\alpha\)T7 loop with helices \(\beta\)H1 and \(\beta\)H2 (Fig. 2c,d).

**Fig. 4 | Effect of Taxol binding to GDP-DCX-MT.** a, Lumenal backbone view of GDP-DCX-MT structure without Taxol, colored by the degree of displacement or as follows: Taxol, green; GTP, yellow; GDP, red. Regions showing the largest displacement are limited to loops directly contacting the drug (arrows). b, Unchanged \(\beta\)E-site before (gray ribbon and nucleotide) and after (green ribbon and red or heteroatom-colored nucleotide) addition of Taxol, shown in the density of the MT lattice containing Taxol; selected residues are shown as sticks colored by heteroatom. c, Close-up on Taxol binding site in GDP-DCX-MT reconstruction with Taxol (green) and without Taxol (gray), focusing on local structural changes. Models are colored as in a; the most displaced regions are shown as sticks where density is present, and highlighted with arrows. Reconstruction without Taxol has weaker density for the R276 side chain (not visible at this density threshold), suggesting that Taxol somewhat orders this residue in Taxol-bound MT. Taxol’s close interaction with the \(\beta\)M loop—a main contributor to MT lateral contacts—explains how the lateral contacts are locked to promote MT lattice stability upon Taxol binding.
in the GTP state model, ~4.6 Å apart in the GDP•Pi state model (Fig. 3b and Supplementary Fig. 5), and ~5.6 Å apart in the GDP state model. Transition 2 also results in noticeable perturbation of lateral contacts between the β-subunits. Of several putative connections (~4 Å) in our models of the GTP and GDP•Pi states (for example, Ala55–Arg282, Lys58–Gln280, Gln83–Tyr281, Arg86–Tyr281, and Glu125–Lys336), separations >4 Å are observed between the β-Ala55–βArg282 and β-Lys58–βGln280 pairs in the GDP state model, while density corresponding to the side chain of β-Lys58 appears distinct in the GDP state reconstruction compared to the GDP•Pi state reconstruction, indicating that this residue is no longer involved in the lateral contact after Pi release (Fig. 3b, Supplementary Fig. 5, and Supplementary Video 4).

**DCX-MT lattice skew varies slightly with nucleotide state.** The GTPase-dependent lattice transitions also manifest as changes in PF skew. The extended GMPCPP lattice shows a slightly right-handed skew (0.16 ± 0.08°). Transition 1 straightens PFS (0.00 ± 0.08°), and transition 2 restores some of the initial skew (0.08 ± 0.07°; Supplementary Fig. 6). The direct influence of DCX on this property is not known, but MTs bound by the end-binding (EB) protein have a left-handed PF skew11, reaching 0.25 ± 0.01° in GTPγS-EB-MT, whereas our GTPγS-DCX-MT is almost straight (~0.02 ± 0.08°; Supplementary Fig. 6 and Supplementary Table 1). Thus, PF skew is influenced by several variables, including the tubulin nucleotide state and bound MAPs, but it is not obvious from these data that it is intrinsic to the mechanism of dynamic instability.

**The role of lateral contacts in MT integrity supported by Taxol stabilization mechanism.** The blockbuster MT-stabilizing drug Taxol binds near the β-subunit lateral contacts and inhibits catastrophes. It has been suggested that it stabilizes MTs by allosterically counteracting lattice compaction. We investigated this idea using GDP-DCX-MTs and found that Taxol binding to these prestabilized MTs did not reverse the GTPase-driven conformational changes. Rather, it locally displaced fragments of the βM and the βS9–S10 loops (≤1.7 Å) that it directly contacts (Fig. 4). Therefore, an alternative explanation of Taxol’s MT-stabilizing effect is that, while tubulin lateral contacts were loosened by GTP hydrolysis (Fig. 3b), Taxol locks the βM lateral loops in the lattice-constrained conformation and thereby prevents PF peeling. Such stabilization of the otherwise labile GDP-MTs may also explain why the drug renders MTs flexible19,20. Since it stabilizes every other lateral contact along each PF (assuming saturation), Taxol probably keeps the PFS only loosely associated within the GDP-MT lattice. Presumably as a consequence, we find MTs prestabilized with Taxol to be poorly decorated with DCX in vitro (Supplementary Fig. 1b). The same is true for cellular MTs11, providing further evidence for looseness of the Taxol-stabilized lattice, which DCX can override if it binds first.

**Discussion**

Our data suggest that a portion of GTP hydrolysis energy is absorbed by the lattice, triggering a two-step loosening of its lateral contacts, first between the α-subunits (transition 1) and then between the β-subunits (transition 2), which coincides with strengthening...
of interdimer longitudinal interfaces (Fig. 5). This explains why whole PFs peel away from MTs after rapid induction of catastrophe. Our data suggest that catastrophe happens because the lateral contacts become too weak to counteract the intrinsic strain of the straight tubulin dimers. The role of homotypic lateral contacts in dynamic instability has been discounted in previous studies10,15, but in comparing MTs stabilized by a single protein ligand (DCX) and in capturing the bona fide GDP•Pi state of the tubulin GTPase, we have identified their likely crucial role in contributing to MT dynamics. Very small differences in the energetics of lateral contact formation due to isoform-specific sequences could be an important mechanism for regulating MT dynamics in particular physiological settings14. As tubulin relaxes (bends), the disassembling PFs curl outward, generating force17,19. The developing curvature in turn weakens the interdimer longitudinal contacts (Supplementary Fig. 7c), allowing complete PF breakdown. So, the fine-tuned MT dynamics seem to only require perturbation of a few connections per dimer to tip the balance toward MT catastrophe. In our GDP-DCX-MT, catastrophe is prevented by DCX stabilization.

These results explain why only GTP- or GMPCCP-tubulin spontaneously nucleate MTs, since only the extended lattice forms favorable lateral contacts, apparently balancing the energetic cost of tubulin straightening. To counteract tubulin bending, the longitudinal contact is also larger in the lattice (−1,533 Å²) compared to X-ray structures of curved PFs (832–1,026 Å²)14,30 (Supplementary Fig. 7c). Only this lattice-imposed strict structural conformation brings the catalytic αE254 residue sufficiently close to the E-site GTP to complete the GTPase machinery (Supplementary Fig. 7d).

The specific flexibility of Taxol-MTs makes sense when the role of lateral contacts in dynamic instability is taken into account. Thus, where Taxol is added to MTs after polymerization has been allowed to proceed, our work suggests that the lateral contacts in these MTs would be loose due to the majority of tubulin being bound to GDP. Taxol works by holding the β-tubulin lateral contacts in place, but the entirety of the lattice is presumably rather flexible, explaining the biophysical properties of these MTs14 and the documented knock-on effects on binding by microtubule regulators, including EBs and DCX15,30. Further, in experiments in which Taxol is added at polymerization initiation, it seems likely that its binding may alter or trap the GTPase-linked structural transitions in some more complex, time-dependent, and structurally heterogeneous way. A previous study14 proposed that Taxol prevents or reverses GTPase-dependent MT lattice compaction and that MT stabilization by the drug relies on preservation of the extended (GTP-like) lattice state. In our study and in another recent publication15, Taxol binding was found to not induce lattice expansion. Similarly, other MT stabilizing agents may also be expected to work by stabilizing lateral contacts in lattice-like conformations.

The stochastic nature of GTP hydrolysis events in the lattice4 would be predicted to cause lateral contact mismatches between neighboring pre- and posthydrolysis dimers. These by themselves could cause tensions in the lattice, destabilizing it and/or synchronizing hydrolysis, presumably contributing to the multistep nature of catastrophe14. The MT seam is an inbuilt source of posthydrolytic lateral mismatch6, but not the only source of MT instability, because MTs do not simply unzip at the seam; instead their individual PFs depolymerize completely. Our analysis shows that the irreversible lattice transitions cause a global MT catastrophe, liberating tubulin to exchange its nucleotide and start the cycle again.

The intrinsic polarity of the tubulin dimers themselves14 and of the conformational changes we have described here (Figs. 2, 3, and 5) likely contribute to the intrinsic but mechanistically poorly understood differences in MT dynamics at the MT (+) and (−) ends. Our data highlight the conformational plasticity of α-tubulin at the longitudinal tubulin interface. If this conformational plasticity is recapitulated at the MT (−) end, we predict that this would provide a previously unanticipated conformational barrier to stable addition of the incoming tubulin dimers. At the (+) end, in contrast, while we predict that α-tubulin retains its conformational plasticity in the incoming free tubulin dimers, the polymer-held β-tubulin would be predicted to provide a more stable platform or template for dimer docking, a stability that is reflected in faster (+)-end growth.

With a multitude of MAPs, polymerases, depolymerases, and modifying enzymes in vivo, MT dynamics are unlikely to be governed by GTP hydrolysis alone, but by a complex network of regulators. For example, recent work has indicated the importance of acetylation around the lateral contacts as cellular means to prolong MT longevity15,20; this reinforces our conclusions about their role in MT stability. Here we show that the majority of MTs—built from GDP•Pi or GDP tubulin—are structurally poised for immediate catastrophe and force generation. However, overriding cellular regulators can ensure that tubulin strain energy remains stored in the lattice until required, prompted by cell physiology.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41594-018-0087-8.

Received: 5 March 2018; Accepted: 1 June 2018; Published online: 2 July 2018

References

1. Mandelkow, E. M., Mandelkow, E. & Mulligan, R. A. Microtubule dynamics and microtubule caps: a time-resolved cryo-electron microscopy study. J. Cell Biol. 114, 977–991 (1991).
2. Rice, L. M., Montabana, E. A. & Agard, D. A. The lattice as allosteric effector: structural studies of alpha/beta- and gamma-tubulin clarify the role of GTP in microtubule assembly. Proc. Natl. Acad. Sci. USA 105, 5578–5583 (2008).
3. Driver, J. W., Geyer, E. A., Bailey, M. E., Rice, L. M. & Asbury, C. L. Direct measurement of conformational strain energy in protofilaments curling outward from disassembling microtubule tips. elife 6, e28433 (2017).
4. Mitchison, T. & Kirschner, M. Dynamic instability of microtubule growth. Nature 312, 237–242 (1984).
5. Cousse, M., Lombillo, V. A. & McIntosh, J. R. Microtubule depolymerization promotes particle and chromosome movement in vitro. J. Cell Biol. 112, 1165–1175 (1991).
6. Nogales, E., Wöf, S. G. & Downing, K. H. Structure of the alpha beta tubulin dimer by electron crystallography. Nature 391, 199–203 (1998).
7. Löwe, J., Li, H., Downing, K. H. & Nogales, E. Refined structure of alpha beta-tubulin at 3.5 Å resolution. J. Mol. Biol. 313, 1045–1057 (2001).
8. Guesdon, A. et al. EB1 interacts with outwardly curved and straight regions of the microtubule lattice. Nat. Cell Biol. 18, 1102–1108 (2016).
9. Alushin, G. M. et al. High-resolution microtubule structures reveal the structural transitions in αβ-tubulin upon GTP hydrolysis. Cell 157, 1117–1129 (2014).
10. Zhang, R., Alushin, G. M., Brown, A. & Nogales, E. Mechanistic origin of microtubule dynamic instability and its modulation by EB proteins. Cell 162, 849–859 (2015).
11. Vale, R. D., Coppin, C. M., Malik, F., Kull, F. J. & Milligan, R. A. Tubulin GTP hydrolysis influences the structure, mechanical properties, and kinesin-driven transport of microtubules. J. Biol. Chem. 269, 23769–23775 (1994).
12. Hyman, A. A., Christen, D., Arna, I. & Wade, R. H. Structural changes accompanying GTP hydrolysis in microtubules: information from a slowly hydrolyzable analogue guanylyl-(alpha,beta)-methylene-diphosphonate. J. Cell Biol. 128, 1102–1108 (1995).
13. Maurer, S. P., Fourniol, F. J., Bohner, G., Moore, R. A. & Surrey, T. EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. Cell 149, 371–382 (2012).
14. Maurer, S. P., Bieling, P., Cope, J., Hoenger, A. & Surrey, T. GTPgammaS microtubules mimic the growing microtubule end structure recognized by end-binding proteins (EBs). Proc. Natl. Acad. Sci. USA 108, 3988–3993 (2011).
15. Maurer, S. P. et al. EB1 accelerates two conformational transitions important for microtubule maturation and dynamics. Curr. Biol. 24, 372–384 (2014).
16. Moores, C. A. et al. Mechanism of microtubule stabilization by doublecortin. Mol. Cell 14, 833–839 (2004).
17. Fourniol, F. J. et al. Template-free 13-protofilament microtubule-MAP assembly visualized at 8 Å resolution. J. Cell Biol. 191, 463–470 (2010).
18. Hyman, A. A., Salser, S., Drechsel, D. N., Unwin, N. & Mitchison, T. J. Role of GTP hydrolysis in microtubule dynamics: information from a slowly hydrolyzable analogue, GMPCPP. *Mol. Biol. Cell* 3, 1155–1167 (1992).

19. Kellogg, E. H. et al. Insights into the distinct mechanisms of action of taxane and non-taxane microtubule stabilizers from cryo-EM structures. *J. Mol. Biol.* 429, 633–646 (2017).

20. Kikumoto, M., Kurachi, M., Tosa, V. & Tashiro, H. Flexural rigidity of individual microtubules measured by a buckling force with optical traps. *Biophys. J.* 90, 1687–1696 (2006).

21. Ettinger, A., van Haren, J., Ribeiro, S. A. & Wittmann, T. Doublecortin is excluded from growing microtubule ends and recognizes the GDP-microtubule lattice. *Curr. Biol.* 26, 1549–1555 (2016).

22. Vemu, A., Atherton, J., Spector, J. O., Moores, C. A. & Roll-Mecak, A. Tubulin isoform composition tunes microtubule dynamics. *Mol. Biol. Cell* 28, 3564–3572 (2017).

23. Zakharov, P. et al. Molecular and mechanical causes of microtubule catastrophe and aging. *Biophys. J.* 109, 2574–2591 (2015).

24. Prota, A. E. et al. Molecular mechanism of action of microtubule-stabilizing anticancer agents. *Science* 339, 587–590 (2013).

25. Wang, Y. et al. Structural insights into the pharmacophore of vinca domain inhibitors of microtubules. *J. Mol. Biol.* 428, 2981–2988 (2016).

26. Prota, A. E. et al. Pironetin binds covalently to αCys316 and perturbs a major loop and helix of α-tubulin to inhibit microtubule formation. *J. Mol. Biol.* 428, 2981–2988 (2016).

27. Navrotek, A., Knossow, M. & Gigant, B. The determinants that govern microtubule assembly from the atomic structure of GTP-tubulin. *J. Mol. Biol.* 412, 35–42 (2011).

28. Bechstedt, S., Lu, K. & Brouhard, G. J. Doublecortin recognizes the longitudinal curvature of the microtubule end and lattice. *Curr. Biol.* 24, 2366–2375 (2014).

Acknowledgements

We thank A. Roberts and M. Steinmetz for invaluable discussions about this work. S.W.M. and C.A.M. are supported by the Medical Research Council, UK (MR/J000973/1 and MR/R000352/1).

Author contributions

S.W.M. conceived experimental strategies, designed and carried out experiments and computations, processed data, interpreted results, and wrote the manuscript; C.A.M. proposed and supervised research, interpreted results, and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41594-018-0087-8.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Methods

Protein preparation. Human doublecortin isoform 2 (DCX, residues 1–360) was cloned into a pNinc2B8ss vector (Structural Genomics Consortium, Oxford, UK), which adds a tobacco etch virus (TEV) protease-cleavable N-terminal His tag to the protein. After expression in BL21 Star (DE3) E. coli (Invitrogen), the cells were sonicated in a lysis buffer (50 mM Na2HPO4, pH 7.2, 300 mM NaCl, 10 mM imidazole, 10% glycerol, 2 mM DTT) supplemented with protease inhibitor cocktail (Complete Cocktail Tablet, Roche/Sigma Aldrich), and the lysates were clarified by centrifugation. Clear lysates were passed through a nickel HisTrap HP column (GE Healthcare) and His-DCX was eluted with a 0- to 250 mM imidazole gradient. To remove the His tag from the DCX protein, we used a His-tagged TEV protease expressed in-house and then removed both His-TEV and the cleaved tag by passage through nickel beads (GE Healthcare). DCX was then captured on a HiTrap SP HP ion-exchange column (GE Healthcare) equilibrated in BRB80 buffer (80 mM PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)), pH 6.8, 1 mM NaCl, 1 mM EGTA, 1 mM MgCl2, 1 mM DTT (dithiothreitol)) and eluted with a 0- to 500 mM imidazole gradient. DCX was then finally purified and desalted by gel filtration through a Superdex 200 size-exclusion column (GE Healthcare) equilibrated in the BRB80 buffer.

Lyophilized bovine brain tubulin was purchased from Cytoskeleton and reconstituted to 100 μM tubulin in BRB80 solution with 1 mM GTP for dynamic MTs (GTP-tubulin) or one of the nucleotide analogs: GMPCPP (guanosine-5’-[(α,β)-methylene]-triphosphate; Jenfa Biosciences; GMPCPP-tubulin) or GTPγS (guanosine 5’-O-(3-thiotriphosphate); Roche; GTPγS-tubulin).

Preparation of cryo-EM samples. To obtain GMPCPP-DCX-MTs with high GMPCPP occupancy, 30 μM GMPCPP-tubulin was cycled twice through 30 min polymerization at room temperature in BRB80 buffer containing 5 μM GMPCPP-tubulin, and depolymerization in cold buffer. In the third polymerization round, 5 μM GMPCPP-tubulin was co-polymerized with 3.5 μM DCX under the same conditions. This polymerization strategy was required to obtain a fully extended 13-PF MT lattice.

GTPγS does not nucleate MTs, hence it cannot be enriched in the lattice through tubulin polymerization and depolymerization cycles, and its occupancy is not known. For GTPγS-DCX-MTs, 5 μM GTPγS-tubulin was co-polymerized for 30 min with 3.5 μM DCX at 37 °C in BRB80 buffer containing 2 mM GTPγPS.

For GDP-DCX-MTs, 5 μM GDP-tubulin was co-polymerized for 30 min with 3.5 μM DCX at 37 °C in BRB80 buffer containing 1 mM GDP. For GDP-DCX-Taxol-MTs, 1 mM Taxol was added after 30 min of polymerization, and the sample was incubated for further 30 min at 37 °C.

In all the above MT preparations, substoichiometric concentration of DCX versus tubulin was used to nucleate MTs with the desired 13-PF architecture, while keeping MT bundling minimal. These MTs were applied directly to glow-discharged grids (FEI/Thermo Fisher Scientific) and incubated there for 1 min at 30 °C and 95% humidity, before finally blotting and plunging freeze in liquid ethane.

For the 10 μM GTP-tubulin sample, 10 μM GDP was mixed with 50 μM DCX in cold BRB80 buffer containing 1 mM GTP and immediately applied to a glow-discharged lacey grid for rapid polymerization directly on the grid inside the Vitrobot, which was set to 30 °C and 95% humidity. After 30 s incubation, the grid was blotted and vitrified as before.

Cryo-EM data collection. Micrographs were acquired on a 300 kV Polara microscope (FEI) combined with a K2 Summit camera (Gatan) operated in counting mode after the energy filter with a 20-eV slit. The magnification at the specimen plane was 35,971×, resulting in a pixel size of 1.39 Å. The dose rate was ~5 e- /pixel/s, corresponding to ~2.6 e- /Å2/s. The total dose on the specimen was ~23.4 e- /Å2, collected over 9 exposures fractionated into 36 movie frames (0.25 s/frame). We used SerialEM software (http://www.cbf.colorado.edu/SerialEM/) to manually collect exposures in the ~0.4–2.5-μm defocus range.

Image processing and 3D reconstruction. We used MotionCor2 29 to globally and locally (25 tiles/image) align movie frames. Using EMAN 1 Boxer 30, we picked MT segments from these drift-corrected image sums. Boxes 652 × 652 pixels spanned ~11 tubulin dimers and were cut along MTs with ~8-dimer overlaps. These segments were subsequently treated as single particle input to Chuf11,12, a custom-designed multiscript processing pipeline using Spider4 and Frealign 30. The initial shape-finding alignment was done in Spider by projection matching to a synthetic 13-PF wild-type MT render filtered to 30 Å. The initial transfer functions (FTF) parameters were estimated with CTFFIND5, and the CTF correction was performed during local refinement within Frealign, producing isotropic 3D reconstructions with pseudohelical symmetry applied 12 times. Independently processed half-maps were combined in Relion 1.4 40 and subjected to its standard postprocessing routine, involving (i) estimation of map resolution based on Fourier Shell Correlation (FSC) between the two half-maps, (ii) computation of the average B-factor based on Guinier plot using the EMFfactor program 30, and (iii) map sharpening using the computed B-factor value. The resolutions of the final maps were estimated using 0.143 FSC cut-off criterion, and the absence of over-fitting was confirmed with a high-resolution noise-substitution test 30 (Table 1; FSCAB = 0.143). Side chains of acidic residues are mostly missing, likely due to their exceptional susceptibility to radiation damage unless they are stabilized by an interaction (for example, D177 H-bonding with GMPCPP; Fig. 1c).

Atomic model refinement. We used a high-resolution cryo-EM model with six GDP-tubulin dimers (PDB 3JAR 31), devoid of E83 chains as a starting point for refinement in all of our cryo-EM density maps. Nucleotides were substituted as necessary with structures downloaded from the Grade Server (http://grade.globalphasing.org/cgi-bin/grade/server.cgi), and DCX density was masked and excluded from the refinements by zoning maps around tubulin structures in UCSF Chimera. Each isolated map was placed in a new unit cell with P1 space group. Ten macrocycles of refinements in real space were carried out at each round using phenix.real_space_refine (http://phenix-online.org/) with default settings (Ramachandran plot, C-β deviations, rotamer and secondary structure restraints). Noncrystallographic symmetry (NCS) group definitions were manually provided as constraints for the related tubulin chains. The program automatically determined weight between data and the restraints to achieve r.m.s. deviations for covariant bonds not greater than 0.01 and for angles not greater than 1.0. Model geometry was evaluated by MolProbity 32 after each round of refinement, and problematic regions in the models were manually corrected in Coot 33. This process was repeated for every structure until satisfactory level of model:map agreement with excellent model stereochemistry were accomplished (Table 1).

Estimation of PF skew in different MT lattices. We calculated the average difference between the assigned φ-angles (rotation angle around MT axis) of the consensus DCX-MT segments cut along individual MTs in each lattice state. These individual values of skew were then averaged over >50 MTs per lattice state (Supplementary Fig. 6). To determine statistical significance between the means we used one-way ANOVA, since s.d. across all datasets were not significantly different by both Brow–Forsythe and Bartlett’s tests, according to Prism 6 (GraphPad). The degree of freedom within each group (residual) amounted to 355 and between the groups to 4, resulting in an F ratio of 68.67 and significance level (P value) of <0.0001.

Estimation of DCX occupancy in different MT lattices. A Fourier-transformed image of any DCX-MT shows a layer line corresponding to a ~4 nm tubulin subunit repeat and a layer line corresponding to a ~8 nm DCX repeat. To estimate the relative DCX occupancy in each lattice state, we averaged power spectra of all the DMX-MT segments in a given state using EMAN 2 and calculated intensities of the 0.125-nm layer lines in relation to the 0.25-nm layer lines. To calculate the error of that ratio for each lattice state, we divided each dataset into three approximately equal subsets and calculated the average ratio and its s.d. (Supplementary Fig. 1b).

Fig. and video preparation. Molecular visualizations in all figures and videos were prepared using UCSF Chimera 34.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Accession codes. Density maps and coordinates were deposited in EMDB and PDB: GMPCPP-DCX-MT, EMDB-3961 and PDB 6EVZ; GDP(PγS)-DCX-MT, EMDB-3962 and PDB 6EVB; GDP-DCX-MT, EMDB-3963 and PDB 6EVE; GDP-DCX-Taxol-MT, EMDB-3964 and PDB 6EVE; GDP-DCX-MT, EMDB-3964 and PDB 6EVE; GDP-DCX-Taxol-MT, EMDB-3965 and PDB 6EWO.

Data availability. The data that support the findings of this study are available from the corresponding authors upon reasonable request.

References
36. Zheng, S. Q. et al. MotionCor2: anisotropic correction of beam-induced motion for improved cryo-electron microscopy. Nat. Methods 14, 331–332 (2017).
37. Ludtke, S. J., Baldwin, P. R. & Chiu, W. EMAN: semiautomated software for high-resolution single-particle reconstructions. J. Struct. Biol. 128, 82–97 (1999).
38. Sindelar, C. V. & Downing, K. H. The beginning of kinesin’s motion for improved cryo-electron microscopy. J. Struct. Biol. 117–125 (2007).
39. Frank, J. et al. SPIDER and WEB: processing and visualization of images in 3D electron microscopy and related fields. J. Struct. Biol. 116, 99–199 (1996).
40. Grigorieff, N. FREAlign: high-resolution refinement of single particle structures. J. Struct. Biol. 157, 117–125 (2007).
41. Mindell, J. A. & Grigorieff, N. Accurate determination of local defocus and specimen tilt in electron microscopy. *J. Struct. Biol.* **142**, 334–347 (2003).
42. Scheres, S. H. W. RELION: implementation of a Bayesian approach to cryo-EM structure determination. *J. Struct. Biol.* **180**, 519–530 (2012).
43. Rosenthal, P. B. & Henderson, R. Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J. Mol. Biol.* **333**, 721–745 (2003).
44. Pettersen, E. F. et al. UCSF Chimerà visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**, 1605–1612 (2004).
45. Chen, V. B. et al. MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 12–21 (2010).
46. Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 486–501 (2010).
Experimental design

1. Sample size
   Describe how sample size was determined.
   Overall dataset size was defined according to standard in the field. For protofilament skew analyses, sample size was determined with reference to the data scatter and the magnitude of the difference between the compared effects.

2. Data exclusions
   Describe any data exclusions.
   In the protofilament skew analyses excluded were only obvious outliers resulting from misalignment of the consecutive microtubule segments due to poor local image quality (noise in a micrograph) or poor microtubule decoration with doublecortin, which serves as a marker for microtubule orientation and is thus essential for correct alignment. Angle assignments resulting in differences of > 1 degree for the consecutive segments were discarded as obviously unreliable.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   Experiments were reproducible according to the sample sizes reported in figure legends. For quantifying the extent of DCX decoration on various MT lattices or lattice spacings with standard deviations, the data were randomly divided into 3 approximately equal non-overlapping groups.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   Samples were grouped based on the microtubule nucleotide state. Only one nucleotide state was targeted within each single experiment.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   Investigators were not blinded to group allocation

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)

- A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly

- A statement indicating how many times each experiment was replicated

- The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)

- A description of any assumptions or corrections, such as an adjustment for multiple comparisons

- The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted

- A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)

- Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

For cryo-EM image processing: MotionCor2, EMAN 1, Spider, Frealign, Relion 1.4, EMBfactor, ResMap. For atomic model refinement: Phenix, Coot. For atomic model interpretation and visualisations: PDBePISA, Chimera. For statistical analyses: Graphpad Prism.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository [e.g. GitHub]. Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

- n.a.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

- n.a.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

- n.a.

b. Describe the method of cell line authentication used.

- n.a.

c. Report whether the cell lines were tested for mycoplasma contamination.

- n.a.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

- n.a.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
   Provide details on animals and/or animal-derived materials used in the study.
   n.a.

Policy information about studies involving human research participants

12. Description of human research participants
   Describe the covariate-relevant population characteristics of the human research participants.
   n.a.