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Microtubule's Conformational Cap

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ABSTRACT. **The molecular mechanisms that allow elongation of the unstable microtubule lattice remain unclear. It is usually thought that the GDP-liganded tubulin lattice is capped by a small layer of GTP- or GDP-P_i-liganded molecules, the so called "GTP-cap". Here, we point-out that the elastic properties of the microtubule lattice cause a difference in stability between the elongating tubulin sheet and the completed microtubule wall. The implications of our observations for microtubule structure and dynamics are discussed.**

Key words: microtubule/tubulin/electron microscopy/computer modelling

Microtubules in the cell, or assembled *in vitro* at low free tubulin concentration, interconvert infrequently between polymerizing and rapidly depolymerizing states (18, 26, 32). This behavior, termed dynamic instability, provides the microtubule cytoskeleton with high lability and is finely regulated throughout the cell cycle, but is exceptional for a polymer. It is fueled by GTP hydrolysis (7, 8, 26), and is achieved by keeping the body of the microtubule in a state poised to depolymerize, but stabilized by a special capping structure at the microtubule's ends. The exact nature of this cap remains unknown.

Elastic model of the microtubule wall

Tubulin molecules seem to undergo large conformational changes during microtubule assembly and disassembly (9, 10, 13, 19, 21, 23, 25, 29). Depolymerization experiments have revealed that microtubules depolymerize by release of short protofilament segments in a highly curved conformation (Fig. 1a; 21, 23, 29). Growing microtubules, on the other hand, display sheets of various lengths, widths, and curvatures at their extremities (Fig. 1b; 10), indicating that microtubule assembly is a two-dimensional assembly process.

We have previously shown that all these observed shapes can be explained as different manifestations of a single phenomenon (20): The wall of the tube that is the microtubule, is an elastic sheet of material with opposing built-in curvatures. The argument goes as follows: The curved conformation of the oligomers released from depolymerizing microtubule ends reveal that the protofilaments in the microtubule wall are intrinsically curved (Fig. 1c). But since they are bound laterally to each other in the microtubule wall, in a sheet that curves strongly laterally to form the tube, the protofilaments are straightened by the forces at play under these circumstances (Fig. 1d), and their intrinsic curvature is not observed, except when they appear individually at depolymerizing microtubule ends, or together, but in less numbers than it takes to form a whole tube. In the latter case, the sheet that they form curves both laterally and longitudinally, but in opposite directions (the technical mathematical term for this situation is that the sheet has negative Gaussian curvature). Thus, the observed curvatures are the result of a tug-of-war between the opposing intrinsic curvatures, and depend on the number of protofilaments in the sheet. The fewer protofilaments, the larger the longitudinal curvature,

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Abbreviations: GMPCPP, guanylyl-(α-β)-methylene-diphosphonate.

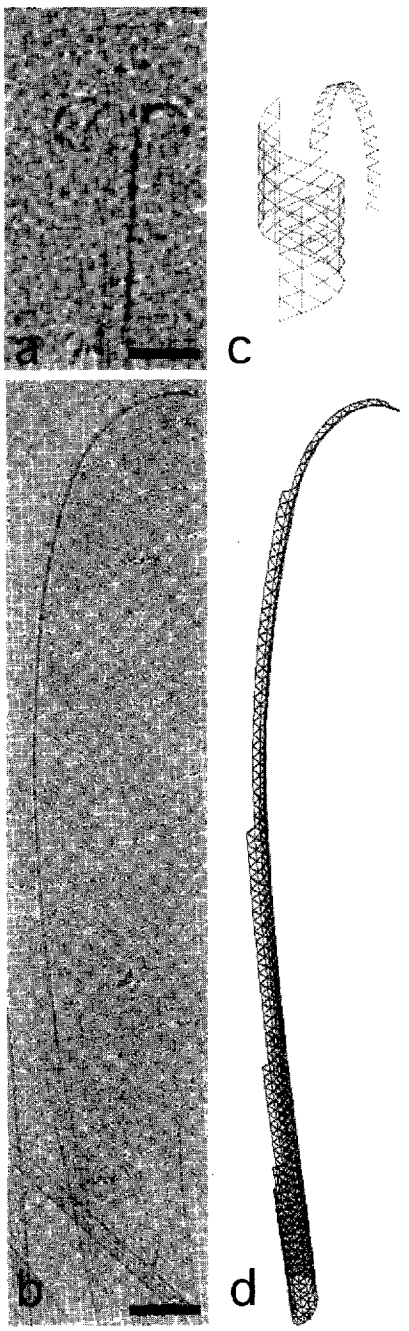

Fig. 1. Microtubule ends observed by cryo-electron microscopy (see Chrétien *et al.*, 1995, for details) and in computer simulations (see Jánosi *et al.*, 1998, for details). (a) Protofilaments peeling off a disassembling microtubule. (b) Outwardly curved sheet at the end of a growing microtubule. The sheet is observed edge-on. (c) Model's description of an individual protofilament. (d) Model's description of a tubulin sheet. The configuration of a given sheet that minimizes its total elastic energy was found with a conjugate-gradient relaxation method. The gradually decreasing width and the physical parameters (bending and stretching rigidities, and intrinsic curvatures) for the model structure in Figure 1d was adjusted to reproduce the observed shape of the sheet in Figure 1b. Scale bars (a) 50 nm, (b) 100 nm.

with a single protofilament displaying the largest curvature, its intrinsic curvature.

Implications for microtubule structure

An interesting consequence of this elastic model for microtubule structure and assembly is that a certain minimum number of protofilaments is necessary to straighten the sheet and curve it laterally to close it into a tube. The lowest number of protofilaments observed in the assembly conditions where the sheets were characterized was 10 (10, 11), and the lowest number reported in the literature is 8 (4). It is likely that sheets composed of fewer protofilaments are unable to close simply because they adopt a gentle outward curvature, as is in fact observed by cryo-electron microscopy (Fig. 1b). Conversely, closure will occur for a preferred protofilament number which must be near 13 or 14, since these are the preferred numbers observed in cells and *in vitro*. Hence, protofilament numbers higher than 13 or 14 are also less likely to occur, because they result in a lateral curvature less than the built-in one. Furthermore, it follows that factors modifying the lateral or longitudinal elastic properties within or between tubulin subunits, as a consequence will change the protofilament number of microtubules as well, like a change in the preferred bond angles will. This is indeed the case, e.g., the antimitotic drug Taxol favors the formation of 12-protofilament microtubules (1, 2), and the GTP analogue GMPCPP favors the formation of 14-protofilament microtubules (19). Add the facts that, although both molecules stabilize microtubules, the former reduces their flexural rigidity (14, 15, 31) while the latter increases it (25). Then these facts and the observed protofilament numbers may all be explained by the built-in curvatures of ordinary tubulin combined with reduced/increased rigidity towards stretching of protofilaments.

Implication for microtubule stability

Another consequence of our model is that the curved sheets must be more stable than the completed microtubule body, independent of their biochemical composition. Each protofilament can be thought of as a spring whose minimal energy configuration is highly curved (Fig. 1a, c). It follows that the change in curvature that occurs during microtubule assembly will induce an accumulation of elastic energy in the microtubule wall. Figure 2 illustrates this process for the tubulin sheet simulated in Figure 1d. Red colors represent stressed regions storing more energy, and blue colors represent more relaxed regions storing less energy. Relating this static result to the growth process of microtubules, we see that the stored elastic energy will

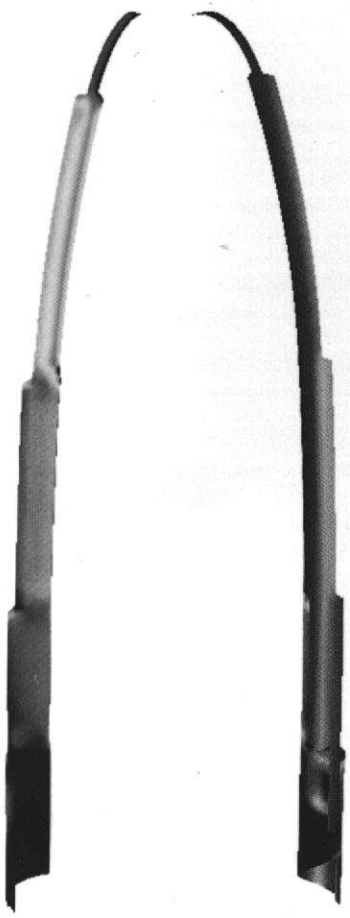

Fig. 2. Color coded representation of the residual elastic energy per unit area stored locally in the microtubule. Views are from the outside (left), and the inside (right) of the sheet. The increase in residual elastic energy from the tip of the sheet (blue) to the microtubule body (red) is a natural consequence of the accompanying increase in protofilament number. The residual elastic energy of the model sheet was obtained by assuming harmonic potentials both for bending and stretching degrees of freedom. Bond-lengths and -angles typically differ from the values minimizing these potentials, even in the configuration of the sheet that minimizes its total energy. This is because its two built-in curvatures cannot be realized simultaneously by any configuration. The residual energy per unit area is larger for wider sheets, and maximal in a full tube. This figure was prepared with SIGMA (Taveau, 1996).

increase as the sheet widens and closes to form the tube. Consequently, the sheet must be more stable than the completed microtubule body, and thus must contribute to the stabilization of the unstable microtubule lattice. Unfortunately, we do not know yet the biochemical composition of the sheets, i.e. whether they are composed of GTP-, GDP-P_i-, or GDP-tubulin molecules. Netherveless, our observations put constraint on the "cap models" that can be proposed.

Implications for the GTP-cap model(s)

GTP-hydrolysis is thought to cause a "straight-to-curved" conformational change in the tubulin molecule (9, 12, 19, 21, 23, 29), with reference to the straight conformation of the tubulin protofilaments in the completed microtubule wall, and to the curved conformation of the tubulin oligomers observed at microtubule ends during depolymerisation. However, a definite proof of the existence of GTP-tubulin subunits at microtubule ends remains to be given. Also, whether GDP-P_i-tubulin subunits can contributes to the stability of microtubule ends is still a matter of debate (6, 24). In the absence of a consensus concerning this matter, we discuss below three possible scenario.

The large GTP/GDP-P_i-cap model

If one speculates that the sheets are essentially composed of GTP- or GDP-P_i-tubulin molecules, it follows logically that these molecules must have an intrinsically "curved" conformation which causes the sheets' curvatures. This hypothesis is clearly at variance from the usual "straight-to-curved" conformational change described above, but is supported by the fact that tubulin molecules liganded with the slowly hydrolyzable GTP analogue GMPCPP form curved oligomers during depolymerization (27). Yet it assembles to form microtubules. Thus the idea that the intrinsic conformation of GTP-tubulin could be curved, does no conflict with the fact that microtubules form from it. Along this line, it is tempting to speculate that GTP-hydrolysis is indeed induced by the "curved-to-straight" forced change of shape that a molecule experiences, when the microtubule grows, and the molecule makes the transition from being part of a curved sheet to being part of a straight microtubule body (10, 25).

The monolayer GTP-cap model

Due to the impossibility of measuring a detectable amount of GTP-tubulin subunits in microtubules, it is widely accepted that if a GTP-cap exists, it is restricted to the tip of the microtubule, possibly to a monolayer of tubulin molecules (3, 22). This idea is supported by the fact that a monolayer of GMPCPP-tubulin molecules is sufficient to stabilize the unstable GDP-tubulin lattice (5, 13). This model would be still compatible with a "straight" conformation of the GTP-liganded tubulin molecule, since these would certainly be undetectable at the end of the curved sheets by cryo-electron microscopy. However, if they do occur there, and play a stabilizing role, we point-out that their only direct effect is on the sheet, while the sheet contributes stability to the end of the microtubule. The model of a mixed GTP-conformational cap proposed by Tran *et al.* (1997b) mostly agrees with this hypothesis.

The conformational-cap model

Finally, could the curved sheets be made exclusively of GDP-liganded subunits? The fact that the sheet length increases with increased microtubule stability is consistent with this hypothesis (10). In addition, severing experiments have revealed that a GDP-tubulin lattice can support elongation (30). The idea of a purely conformational cap is attractive, because it may account for the mechanisms of nucleation and rescue. Microtubule self-assembly is an energetically unfavorable process which involves the initial formation of a nucleus composed, it seems, of 15 to 18 tubulin molecules (16, 17). These numbers may correspond to the minimum number of molecules required to form a curved sheet that can develop into a microtubule, and whose characteristic size is determined by its elastic properties. Along the same line, a so-called rescue from the depolymerizing state could occur if a few protofilaments remain attached or interact laterally during fast disassembly of the microtubule. According to this scenario, microtubule nucleation, assembly, and rescue would share the same basic mechanisms, while the random events causing catastrophe, the transition to the depolymerizing state, remain unknown. But whatever the explanation of catastrophe turns out to be, it is likely that the mechanism of microtubule assembly and mechanical stability properties of the microtubule wall are important factors.

Conclusions

Most previous studies have focussed on the biochemical reactions that accompany microtubule assembly, and the role of GTP-hydrolysis in destabilizing the microtubule lattice has been clearly established. However, the nature of the cap and the molecular events at the origin of catastrophes and rescues remain largely unknown. We propose that dynamic instability results not only from the biochemical properties of the tubulin molecule, but also from the mechanical properties of the microtubule wall. These observations should lead to new experiments aimed at understanding the molecular basis of microtubule dynamic instability *in vitro* and in cells.

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