Published in final edited form as:

J Mol Biol. 2009 November 27; 394(2): 177–182. doi:10.1016/j.jmb.2009.09.033.

Lattice Structure of Cytoplasmic Microtubules in a Cultured Mammalian Cell

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Abstract

Tubulin can polymerize in two distinct arrangements: “B-lattices” in which the α -tubulins of one protofilament lie next to α 's in the neighboring protofilaments; or the “A” configuration, where α 's lie beside β 's. Microtubules in flagellar axonemes and those assembled from pure tubulin in vitro display only B-lattices, but recent work shows that A-lattices are found when tubulin co-polymerizes in vitro with an allele of EB1 that lacks C-terminal sequences. This observation suggests that cytoplasmic microtubules, which form in the presence of this “tip-associating protein,” may have A-lattices. To test this hypothesis we have decorated interphase microtubules in 3T3 cells with monomeric motor domains from the kinesin-like protein, Eg5. These microtubules show only B-lattices, as confirmed by visual inspection of electron cryo-tomograms and power spectra of single projection views, imaged at higher electron dose. This result is significant because 13 protofilament microtubules with B-lattices must include a “seam”, one lateral domain where adjacent dimers are in the A-configuration. It follows that cytoplasmic microtubules are not cylindrically symmetric; they have two distinct faces, which may influence the binding patterns of functionally significant microtubule-interacting proteins.

Introduction

Microtubules (MTs) in most cells are formed from 13 parallel strands of tubulin heterodimers called protofilaments. Neighboring protofilaments are registered so adjacent tubulin monomers form a left-handed, three-start helix. This geometry can be built in two ways (1): with each α -tubulin lying almost beside a β -tubulin (staggered by 0.9 nm), forming the “A-lattice”, or with each α - lying beside another α , making the “B-lattice” (Figure 1). Early workers favored the A-lattice, in part because of structural evidence from A-sub-tubules in the doublets of flagellar

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axonemes (1). The incomplete B-sub-tubules of doublets showed a B-lattice, but the helical symmetry of the A-lattice suggested a pleasing simplicity for all other microtubules, including those found in cytoplasm. Each 13-protofilament MT with a B-lattice must contain one pair of protofilaments that lies in the A-configuration, a singularity that is a priori unexpected (2,3).

Direct determination of MT structure to discriminate between these lattices was initially difficult, because the tubulin isoforms are so similar they are hard to distinguish in the electron microscope. Later, it was realized that catalytic domains of kinesins can be used as visible markers for the tubulin dimer lattice, because the motor heads bind predominantly to β -tubulin (4). With this method, both flagellar MTs and MTs formed in vitro displayed a B-lattice; only one pair of protofilaments per MT was in the A-configuration, as visualized by freeze-fracturing (5). This special boundary between protofilaments was called a “seam”, and MT structure was taken as solved (6). Confirmation of the B-lattice was later obtained through detailed study of MTs interacting with End-Binding protein 1 (EB1), which binds MTs at or near their growing plus ends, both in vivo and in vitro (7-9). High-resolution metal shadowing demonstrated that EB1 binds to a single line between one pair of adjacent protofilaments, suggesting that it binds preferentially to the A-lattice found at the seam (10).

Recently, however, the universality of the B-lattice has been challenged. When MTs are formed in vitro through co-polymerization of tubulin and an allele of EB1, A-lattice contacts are favored (11). Most likely, the frequency of A-lattice contacts is increased because EB1 prefers to bind tubulin with an A-lattice arrangement, and the additional bonding energy from tubulin-EB1 interaction favors this tubulin configuration (12). Because MTs in cells are assembled in the presence of EB1 and many other MT-associated proteins, the preference of tubulin-EB1 for the A-lattice might define MT structure in vivo. The debate about lattice structure for cytoplasmic MTs has therefore been re-opened. The distinction is important, because of the unique line along the surfaces of B-lattice MTs. Just as this domain prefers EB1, providing MT stabilization in a rather economical way (10), a seam could define binding sites for other functionally significant molecules.

Methods and Results

Cell Lysis Can Preserve Cytoplasmic Microtubules and Allow Motor Binding

To visualize the dimer lattice of cytoplasmic MTs in vivo, we perfused lysed cells with monomeric motor domains from the kinesin-like protein, Eg5 (13). 3T3 cells were cultured by conventional methods on either glass coverslips (for light microscopy) or carbon-coated electron microscope grids with regularly arranged holes (Quantifoil grids, EMS, Hatfield, PA). We used immunofluorescence with anti-tubulin and/or anti-Eg5 to identify conditions that would preserve cytoplasmic MTs during a lysis that was sufficient to allow the entry of motor heads, so they could bind to the cell's MTs. A buffer containing 0.1% Triton X-100 in 60 mM Pipes, 25 mM Hepes, 10 mM EGTA, and 2 mM $MgCl_2$, pH 6.9 (14) gave adequate preservation of MTs for more than 1 min, but 20 sec. was sufficient to allow a considerable amount of Eg5 to enter the lysing cells and bind to cytoplasmic MTs (Figure 2). We therefore grew cells on electron microscope grids, lysed them for 30 sec under these conditions, blotted the grids with filter paper, and froze them rapidly by plunging into liquid ethane. Samples for electron tomography were supplemented by the addition of a 1 μ l drop containing 10 nm colloidal gold (British Biocell, International) about 10 sec before blotting and freezing.

Motor-Decorated MTs Reveal the Tubulin Lattice

Grids bearing lysed, motor-decorated, frozen-hydrated cells were scanned at $\sim -180^\circ C$ in a Tecnai-F30 electron microscope equipped with a Compustage (FEI-Company, Eindhoven, NL) and a tilting cryo-rod (Gatan, Inc., Pleasanton, CA). Regions where the thin margins of

cells spanned holes in the carbon film were identified, and those where the ice was sufficiently thin were imaged, either as a single frame of a $2K \times 2K$ charge-coupled device camera (Gatan, Inc.), dose = ~ 30 electrons/ $\text{\AA}^2$, or as tilt-series comprised of about 60 images at 2° intervals from -60° to $+60^\circ$, recorded through a Gatan Imaging Filter operating in the zero-loss mode (slit width = 10 eV) onto a Gatan Ultracam, lens-coupled camera. Total electron dose was $100 \text{ e}/\text{\AA}^2$, sufficient to allow alignment of the tilted views and reconstruction by back-projection, using the IMOD software (15).

Virtually all of the cytoplasmic MTs in these lysed, interphase cells were heavily decorated by motor domains (Figures 2, 3, 4). The Eg5 heads were always distributed in a left-handed, 1.5-start helix, which we presume reflects the underlying lattice of tubulin dimers. We studied 43 MTs in 6 cryo-tomograms (as shown in Figure 3); B-lattices were seen over a total length of $53 \mu\text{m}$, and no A-lattices were detected. A QuickTime movie constructed from a representative tomogram is available in Supplementary Material. By examining successive tomographic slices in real time, one sees clearly the arrangement of the motor heads associated with the MT surface.

We also looked at 42 different fields from interphase cells imaged with a single projection at higher electron dose. Thirty-one of these contained ice that was thin enough to permit clear visualization of the MT lattice. In these views we found 85 distinct MTs, on which we could see a total of about $130 \mu\text{m}$ of MT length. All these images were consistent with a B-lattice, not an A: they all showed clear 8 nm periodicities along the MTs lateral edges, and some showed clear cross-striations at a slight angle to the MT perpendicular, indicative of the B-lattice arrangement of MT-associated motor heads. Computed power spectra were obtained from seven of these MTs, and the reflections seen were compatible almost exclusively with a B-lattice configuration (Figure 4). Some layerlines do include additional intensities, but these seem to derive from the visual noise that is common in images of biochemically complex samples (*vis* the background structures in Figs. 3 and 4) as well as from the overlapping of MTs that was sometimes seen. No evidence for a clear A-lattice was detected. Moreover, most of the MTs in our images showed convincing evidence for straight, paraxial protofilaments, a hallmark of MTs built from 13 protofilaments, the number most commonly seen in MTs *in vivo*.

Discussion

These results extend previous findings by showing that most if not all MTs of interphase 3T3 cells are built with B-lattices, even though these MTs were formed *in vivo*, and thus in the presence of EB1 and other MT-associated proteins. If B-lattices are common in this cell type, it seems likely that most if not all cytoplasmic MTs will have similar lattices, and thus contain seams. This result is certainly consistent with the bulk of evidence about the structure of MTs formed *in vitro* and of axonemal MTs from cells (5,6); it argues against the hypothesis recently proposed that the interactions between tubulin and end-binding proteins might alter the minimum energy form of tubulin assembly in cells more generally (11).

If cytoplasmic MTs are built from 13 protofilaments arranged in a B-lattice, they are not helically symmetric. Their seam defines a line parallel to the MT axis where adjacent tubulins are arranged differently from all other tubulins on the MT surface, because the tubulins on either side of this seam meet their neighbors with the A-lattice configuration. As a result, they might be able to bind MT-associating proteins in a unique way. This tubulin arrangement has been proposed to explain the preference of EB1 for a single, paraxial line on MTs forming *in vitro* (10). The same logic may pertain for other MT-associating proteins, like motor enzymes. Motor heads can certainly bind tubulin dimers all over the MT surface, but the tails of some motors might have a preference for binding at a seam. This situation would endow motor-MT

complexes with an important physiological property: they could walk over neighboring, parallel MTs. Cylindrically symmetric MTs cannot generate a net force on parallel neighbors, because whenever a given MT pushes down on one of its parallel neighbors, the second MT should (by symmetry) push down on it, leading to a net force of zero. Only when cylindrical MTs are antiparallel can they generate a net sliding force by pushing on each other (16). In cells there are parallel arrays of MTs that do generate sliding forces, allowing them to telescope apart (17); the presence of B-lattices in vivo rationalizes this otherwise puzzling observation.

If tubulin-EB1 complexes behaved in vivo as they do in vitro (11), one would have expected at least some cytoplasmic MT with an A-lattice, or perhaps many showing evidence a “mixed lattice” (part A and part B); our results, however, showed almost exclusively B-lattices. This observation suggests that factors other than the minimum energy association of neighboring proteins (e.g., tubulins, EB1, and perhaps other MT-associating proteins) control the geometry of tubulin polymerizing in vivo. This draws attention to the mode by which cytoplasmic MTs are initiated. Probably the gamma-tubulin ring complex defines the organization of the tubulin lattice that assembles upon it; if γ -tubulin binds the minus end of α -tubulin more strongly than the plus end of β , the seeds that initiate MTs in vivo will specify lattice geometry as well as MT polarity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by grants from the NIH to JRM (GM033787), AH (RR000592), and SPG (GM054141).

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Abbreviations Used

MT	microtubule
EB1	end-binding 1

Figure 1.

Comparison of A- and B-lattices for MTs. Upper panels show the lattices unfolded to form flat sheets; lower panels show tubular images and sketches of their power spectra. **(A)** In the B-lattice protofilaments are laterally arranged so adjacent α -tubulins show an axial stagger of 0.9 nm, forming a left-handed 3-start helix of monomers, or a 1.5-start helix when dimers are taken as the axially repeating unit. A 13-protofilament B-lattice must contain one set of A-lattice contacts, commonly called a seam. This disrupts helical symmetry. **(B)** In the A-lattice the major lateral contacts are between α - and β -tubulin, so dimers show a 4.9 nm stagger. The dominant helical arrangements of dimers are a right-handed 5-start and a left-handed 8-start helix. Schematic power spectra are shown at right in the lower panels. Reflections from only one surface of the tube are shown. In these patterns the equator is at the bottom; above it are the 1/8 nm and 1/4 nm layerlines. Dominant peaks are shown in black.

Figure 2.

Immunofluorescence of cytoplasmic MTs decorated with the monomeric motor domain of a kinesin 5. Mouse 3T3 cells were either (A) fixed for 10 min with 4% paraformaldehyde in phosphate-buffered saline and then permeabilized with 0.1% Triton X-100/PBS for 2 min or (B-D) fixed in the same way after 20 sec of lysis with 0.1% Triton X-100 in the Pipes/Hepes buffer described in Materials and Methods. Eg5 motor plus 1mM AMP-PNP were added to the lysis buffer in B and D. Cells were then stained either with anti-tubulin followed by Alexa Fluor[®] 594 goat anti-mouse antibodies (A and B) or anti-Eg5 followed by Alexa Fluor[®] 488 goat anti-rabbit antibodies (C and D). Nuclei were stained with DAPI (blue). Scale bar = 20 μ m.

Figure 3.

Electron cryo-tomography of Eg5-decorated cytoplasmic MTs. **A** (top and bottom) show slices cut at different levels through this representative cryo-tomogram of motor decorated MTs. In the upper image the sampling plane cuts one MT near its axis (black line on diagrammatic insert); in the bottom image, sampling is near the MT periphery, so the bound motor heads are evident. Their arrangement is characteristic of a B-lattice. The images in **B-D** illustrate this point with more detail, displaying a MT that shows an obvious seam. In **B** upper, the “top” surface of this MT is seen. The area boxed by a dashed red line is shown below (“towards viewer”) with red lines marking the 1.5-fold helix of motors adsorbed to the tubulin lattice and a dashed yellow line demarking the seam. **C** shows a slice cut near the center of this MT, and **D** displays a slice near the MT “bottom”, where the helix of motors has the opposite tilt (green lines in lower image, “away from viewer”). These geometrical features of a decorated MT surface unambiguously demonstrate a B-lattice. Note that MT decorations at the orientation of the red lines are visible in many regions of **A**. The QuickTime movie of this tomogram (supplementary materials) makes the point even more clearly.

Figure 4.

Cryomicrographs of frozen-hydrated, Eg5-decorated, cytoplasmic MTs, imaged at higher electron dose, together with the power spectra of selected regions. **A – C** show three distinct fields containing six MTs whose power spectra are shown with the corresponding numbers at right. The equators of these patterns are vertical and the meridians are located near the bottom edge of each image. In **D** both MTs and decorated tubulin sheets, or “C-MTs”, are seen. The power spectra of all these tubulin assemblies show strong peaks on the 1/4 and 1/8 nm layerlines at Bessel orders of -2 and -4, respectively. The peak at Bessel order -1.5 is characteristic of a B-lattice arrangement. Tubulin sheets in D show rather elongated 1/8 and 1/4 nm peaks, indicating an intrinsic curvature tending towards a tube, despite the open sheet configuration.

On sheets the dominance of the B-lattice is clearly visible in the micrograph, due to the angle between the microtubule axis and the striations formed by the motor domain alignments.