



CYTOSKELETON NEWS

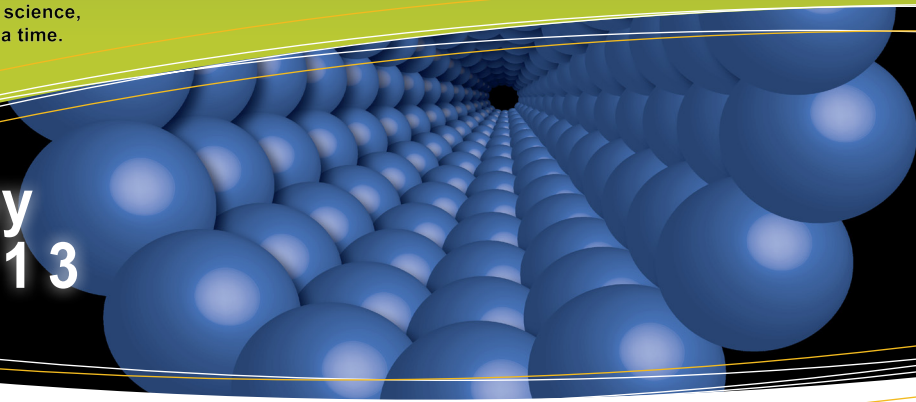
NEWS FROM CYTOSKELETON INC.

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Life Inside a Microtubule

Microtubules (MTs) are dynamic cytoskeletal structures with multiple functions in cell growth, division, and morphological change. This review focuses on the MT lumen as a possible functional entity. The internal environment of the MT has its own peculiar biophysical state and is largely thought to be excluded from cytoplasmic influence, except for the 2 nm² lateral pores¹ and two 200 nm² entrances at its ends^{2,3}. Its biophysical state is outside the scope of this article, but it has very interesting vitreous, electromagnetic resonance, and optical properties⁴.

Several groups have expanded our view of what life is like inside MTs. The first of these breakthroughs came in the 1960s and 1970s by studying the structure of MTs at nm resolution with electron microscopy. Among other observations of MTs, researchers found evidence of 4-7 nm spherical particles in the lumen of MTs of highly fixed cells⁵⁻⁸. The particles' existence and frequency varied between cell type, with neuronal cells having the most particles. Also, Burton⁹ found the particles could be voided from the lumen by rapid disassembly and re-assembly of intracellular MTs. Similar observations over the course of the next 30 years have culminated with observation of these particles in the MT lumen of non-fixed frozen sections of cells using vitreous cryoelectron microscopy or cryoelectron tomography¹⁰. Identification of intraluminal components has been difficult, but recent reports have found evidence of tubulin binding proteins and tubulin modifying enzymes.

Tubulin acetyltransferase (TAT) is known to transfer an acetyl group from acetyl-CoA to the luminal side of stable MTs at Lys40 of alpha-tubulin¹¹. Although it is thought that luminal acetylation does not influence MT stability, it has been associated with differential binding of MT-associated proteins (MAPs), kinesin motor affinity (involving MT surface binding site^{12,13}, but this was recently disputed by two groups^{11,14}), MT severing¹⁵, and increased MT stiffness which is useful in mechanosensory and cilia functions^{16,17}. Whether the TATs and tubulin histone deacetylase enzymes (HDAC6 and Sirt2) are a component of the 4-7 nm particles described above remains to be determined. However, the single or dimer form of TAT

could possibly fill the void (TAT is a 3 x 6 x 3 nm ovoid¹⁸) and there is evidence of MEC-17 (a *C. elegans* TAT homolog) being localized within the MT lumen and in close association with the inner MT wall¹⁶. Tau, a major neuronal MAP, is also known to have a binding site within the MT lumen¹⁹.

How do proteins and particles get into the MT lumen? There are the obvious entry points such as those mentioned in the first paragraph (2 nm² pores and 200 nm² ends). However, each have limitations, as the 2 nm² pores' access is limited to small molecules (<1000 Da^{20,21}) or thin strand-like molecules; for the MT ends, access is limited by the distance molecules can travel by diffusion²². A MT's mid-point can be upwards of 40 μm away from the MT end, and theoretical measurements put the diffusion rate of a 50 kDa protein entering an end between minutes/40 μm to years/40 μm, depending on the molecule's affinity for internal MT walls²². Less obvious entry points include frayed MT ends^{10,23} and growing MT ends in conjunction with MT treadmilling^{24,25}, and the breathing walls of the MT lattice recently observed by vitreous cryoelectron microscopy¹⁷ (see Fig. 1).

Entry points into the microtubule lumen

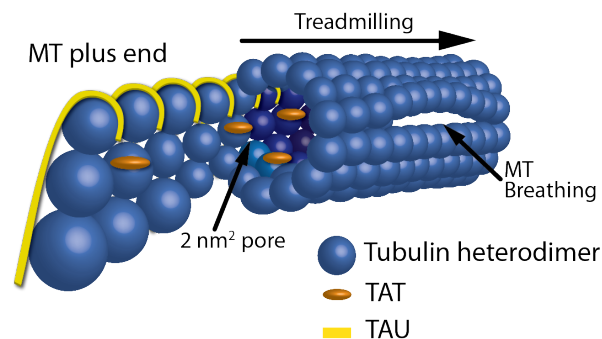


Figure 1: Schematic representation of the entry points into the MT lumen. Showing, from left to right, a frayed/growing MT plus end capturing TAT and tau molecules, treadmilling, 2 nm² pores, a 200 nm² open MT plus end, and a breathing MT lattice.

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Of the less obvious entry points, irregular or curling frayed MT ends have been observed in growing and shortening MTs *in vitro*, and recently *in vivo* via vitreous cryoelectron microscopy¹⁹ and digital model convoluted fluorescence microscopy²³. When protofilaments are exposed at plus ends of MTs, the future MT lumen is exposed and possibly can act as a capture point for proteins/particles destined for the lumen. Subsequent treadmilling could allow the captured entities to move to the minus end of the MT at 1 to 60 $\mu\text{m}/\text{min}$ ²⁵. This is one possible mechanism to explain why the MAP tau has a binding site in the lumen as well as on the surface of MTs when MTs are formed in the presence of tau, but not when tau is added to pre-formed MTs which only results in tau binding to the outside surface of MTs¹⁹. Thus, in practical experiments to show molecules captured inside growing MTs, molecules can be included in a reaction containing polymerizing tubulin as compared to using pre-formed MTs (for example, compare Cat. # BK029 versus Cat. # T240 datasheet method sections, see below).

In summary, several recent key papers indicate important molecules reside inside MTs, including acetylated alpha tubulin, TATS, the two major MT stabilizing agent (MSAs) binding sites of taxol and laulimalide/peloruside²⁶, and of course, tau protein. In addition to the incredible biophysical properties recently reported by Sahu *et al.*⁴, these observations indicate life inside a MT is turning out to be just as interesting as on the MT surface.

Microtubule Related Research Tools

Protein	Source	Purity	Cat. #	Amount
Microtubules pre-formed, lyophilized	Bovine Brain	>99%	MT001-A MT001-B	4 x 500 μg 1 x 10 mg
Microtubules pre-formed, lyophilized	Porcine Brain	>99%	MT002-A MT002-B	4 x 500 μg 1 x 10 mg
Tubulin Protein Lyophilized (no glycerol)	Porcine Brain	>99%	T240-A T240-B	1 x 1 mg 5 x 1 mg
Tubulin Protein, MAP rich	Porcine Brain	70% tubulin 30% MAPs	ML116-A ML116-B	1 x 1 mg 5 x 1 mg
Tau Protein	Bovine Brain	>90%	TA01-A TA01-B	1 x 50 μg 3 x 50 μg
Microtubule Associated Protein (MAP) Fraction	Bovine Brain	70% MAP2	MAPF-A MAPF-B	1 x 100 μg 5 x 100 μg
Tubulin for HTS Applications	Porcine Brain	97%	HTS03-A HTS03-B	1 x 4 mg 1 x 40 mg

Kit	Cat. #	Amount
Microtubule / Tubulin <i>In Vivo</i> Assay Biochem Kit™	BK038	30-100 assays
Microtubule Binding Protein Spin-Down Assay Biochem Kit™	BK029	30-100 assays
Tubulin Polymerization Assay Biochem Kit™ Turbidometric-based, >99% pure tubulin	BK006P	24-30 assays
Tubulin Polymerization Assay Biochem Kit™ Turbidometric-based, >97% pure tubulin	BK004P	24-30 assays
Tubulin Polymerization Assay Biochem Kit™ Fluorescence-based, >99% pure tubulin	BK011P	96 assays

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