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Microtubule Stabilization by the Post-translational Modification of Acetylation

The acetylation post-translational modification (PTM) of tubulin occurs primarily on the lysine at position 40 (Lys40) on the alpha-tubulins of assembled microtubules (MTs)¹⁻³. The predominant alpha-tubulin acetyltransferase is TAT1/MEC-17^{4,5}. Deacetylation of acetylated tubulin is mediated by histone deacetylase 6 (HDAC6) or SIRT2, the mammalian homolog of silent information regulator 2/sirtuin type 2^{6,7}. Lys40 has long been considered the sole site of acetylation and while other lysine residues on alpha-tubulin and beta-tubulin are targets for acetylation based on proteomic analyses, functional studies focus on the Lys40 residue within the MT lumen^{2,8,9}. This newsletter discusses tubulin acetylation, MT stability, and the functionality of acetylated MTs (Fig. 1).

Acetylated MTs and Stability

Acetylation is a marker for stabilized, long-lived MTs (defined as MTs resistant to nocodazole- and colchicine-induced depolymerization) with a half-life of hours¹⁰. Notably, acetylation itself does not cause MT stabilization¹⁰⁻¹⁵. However, a recent genetic ablation study strongly supports the conclusion that Lys40 acetylation is required for maintaining long-lived,

stable MTs in mammalian cells. Loss of TAT1 resulted in a loss of stable, acetylated MTs that are normally present after nocodazole treatment eliminates dynamic MTs¹⁶. Conversely, TAT1 overexpression significantly increased the amount of nocodazole-resistant (stable) MTs and fibroblasts lacking the tubulin deacetylase HDAC6 have more nocodazole-resistant MTs. The dogma that acetylation is restricted to stable MTs has been revised in recent years as tubulin acetylation is also found on subpopulations of dynamic MTs (half-life of minutes)¹⁴, such as those found in both young and mature neurons^{6,14,15}.

Functional Significance of Acetylated, Stable MTs

Acetylated, stable MTs are found in various cell types, comprise many different cellular structures¹⁴, and have a role in multiple physiological cellular functions. These include ciliogenesis, cell cycling, and cell signaling¹⁴, as well as roles in neuron development and migration, synaptic targeting of proteins, and kinesin and dynein motor binding and activity^{6,14}. In addition, acetylated MTs are implicated in neuron function/dysfunction and intracellular transport in neurodegenerative diseases¹⁴.

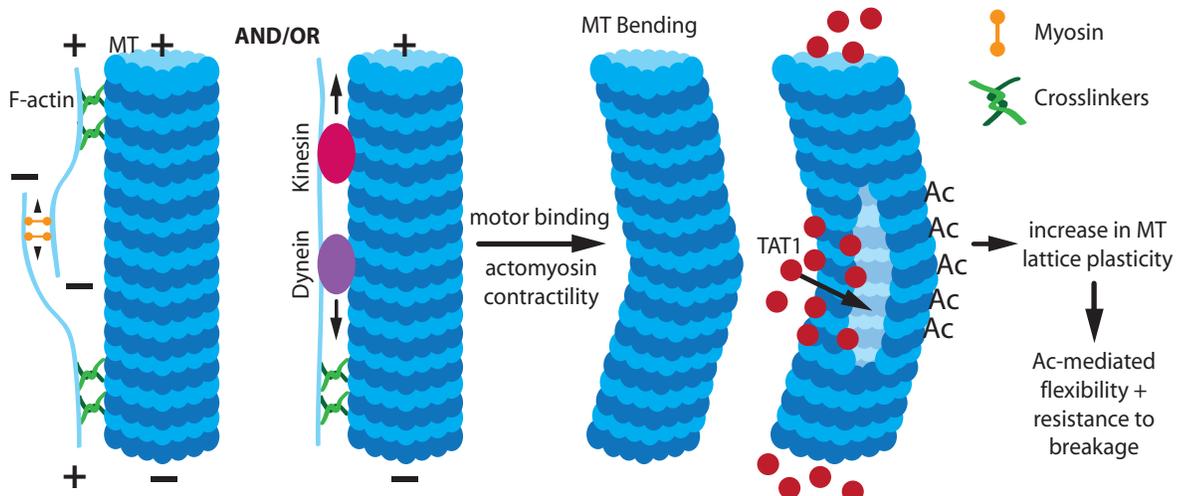


Figure 1. Acetylation (Ac)-induced MT stabilization. Actomyosin contractility and/or motor binding causes mechanical stress, leading to MT bending which allows TAT1 entry. TAT1 acetylates lysine residues, which increases MT lattice plasticity and confers increased MT flexibility and resistance to breakage.



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Despite all of these physiological studies, the functional significance of acetylating long-lived, stable MTs remains undefined. There are conflicting results regarding the role, if any, acetylation has in tubulin polymerization. An early study found no effect of acetylation on depolymerization or polymerization¹⁷. Similarly, loss of TAT1 in retinal pigment epithelial cells did not significantly affect MT polymerization or organization¹⁶. Alternatively, a recent study¹⁸ found acetylated tubulin heterodimers display a decreased rate of spontaneous nucleation, resulting in self-assembly significantly slower than deacetylated tubulin¹⁸. Single MT dynamics revealed that acetylation conferred no change in growth rate, but acetylated MTs depolymerized faster than deacetylated MTs¹⁸. The changes in nucleation and self-assembly were due to acetylation-stimulated disruption of lateral interactions between protofilaments¹⁸, which resulted in reduced nucleation rates and accelerated shrinkage (depolymerization). Acetylation-mediated effects on protofilament interactions underlie the role acetylation has in the response of stable MTs to mechanical stress (Fig. 1).

Long-lived, stable MTs undergo buckling due to mechanical stresses such as compression from binding of kinesin and/or dynein motors and actomyosin contractility^{19,20} (Fig. 1). Acetylation protects MTs from mechanical stress *in vitro*¹⁸, suggesting that localized acetylation protects stable MTs from mechanical stresses such as bending under compression¹⁶. In TAT1-deficient cells, long-lived MTs were reduced due to increased breakage under mechanical stress. The primary source of compression was from Rho/ROCK-mediated actomyosin contractility following nocodazole-induced MT depolymerization^{16,21}; motor binding was also a factor. Acetylation mechanically stabilizes MT through a reduction in MT flexural rigidity (i.e., bending resistance) through a weakening of inter-protofilament interactions¹⁸. This increases the plasticity of the MT lattice, thereby reducing the extent of damage induced by repeated mechanical stress (Fig. 1). In MT segments that bend, there are lattice openings and under mechanical stress/compression, an opening (or openings) may develop that allow TAT1 entry into the MT lumen where acetylation of alpha-tubulin within the bend (i.e., region of MT undergoing stress) occurs, which protects the MT at this vulnerable point^{16,22}. Once inside the MT lumen, the mobility of TAT1 is controlled by TAT1 affinity for acetylation target sites and their corresponding accessibility and localization within the lumen²² (Fig. 1).

Summary

Tubulin acetylation is found within a wide variety of cellular structures and cell types and has been implicated in a myriad of complex cellular functions, including the response to mechanical stresses applied to stable MTs. Cytoskeleton Inc., offers reagents for studying the levels of endogenous PTMs such as acetylation within cells in a sensitive and quantitative manner using the Signal Seeker™ Acetyl-Lysine Enrichment Kit. In addition, there are Enrichment Kits for tyrosine phosphorylation, SUMOylation, and ubiquitination. For more information, please contact one of Cytoskeleton's technical support scientists at signalseeker@cytoskeleton.com or tservice@cytoskeleton.com.

Signal Seeker™ Kits

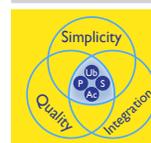
Kit or Affinity Beads	Type	Reactions	Cat. #
Signal-Seeker™ Acetyl-Lysine Enrichment Kit	Kit	30	BK163
		10	BK163-S
Signal-Seeker™ Phosphotyrosine Enrichment Kit	Kit	30	BK160
		10	BK160-S
Signal-Seeker™ Ubiquitin Enrichment Kit	Kit	30	BK161
		10	BK161-S
Signal-Seeker™ SUMO 2/3 Enrichment Kit	Kit	30	BK162
		10	BK162-S

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PTM Antibodies, Beads, Etc

Kit or Affinity Beads	Type	Size	Cat. #
Acetyl-Lysine Antibody Mouse Monoclonal (7B5A1)	MAB	2 x 100 ul	AAC02
		1 x 25 ul	AAC02-S
Acetyl-Lysine Antibody Mouse Monoclonal (19C4B2.1)	MAB	2 x 100 ul	AAC03
		1 x 25ul	AAC03-S
Acetyl-Lysine-HRP Antibody Mouse Monoclonal (19C4B2.1)	MAB	1 x 100 ul	AAC03-HRP
		1 x 25 ul	AAC03-HRP-S
Acetyl-Lysine Affinity Beads	Beads	4 x 500 ul	AAC04-beads
Phosphotyrosine Affinity Beads	Beads	4 x 330 ul	APY03-beads
SUMO 2/3 Affinity Beads	Beads	2 x 400 ul	ASM24-beads
Ubiquitin Affinity Beads	Beads	40 reactions	UBA01-beads
Control for IgG Beads	Beads	10 reactions	CIG01-beads
Control for Ubiquitin Affinity Beads	Beads	10 reactions	CUB02-beads



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