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Polymodifications of tubulin: Glutamylation and Glycylation

Post-translational modifications (PTMs) are highly dynamic and often reversible processes where a protein's functional properties are altered by addition of a chemical group to its amino acid residues. As a major cytoskeletal protein with roles in cell development, growth, motility, and intracellular trafficking, microtubules (MTs) are a major substrate for PTMs. Tubulin PTMs usually occur post-polymerization and preferentially on the α/β tubulin heterodimers of stable (vs dynamic) MTs[1-3]. Two such PTMs are polyglutamylation and polyglycylation, the addition of one or more glutamate or glycine residues, respectively[4-5]. The MTs that comprise the mitotic spindle, neuronal projections, centrioles/basal bodies, and axenomes undergo polyglutamylation (Fig. 1). Conversely, polyglycylation occurs mainly on MTs of the axoneme, the cytoskeletal structure that comprises flagella and cilia (Fig. 2). Flagella are specialized organelles that protrude from a cell's surface, including cells with a primary role in human reproduction. Structurally similar, cilia protrude from the surface of most cells to mediate cell locomotion, flow generation and responses to external stimuli[6-8]. Recently, abnormal formation or function of cilia has been linked to a broad range of human genetic disorders termed ciliopathies[6]. Given the prominent roles that tubulin glutamylation and glycylation play in cilia function (see below), these polymodifications may be involved in a myriad of human diseases.

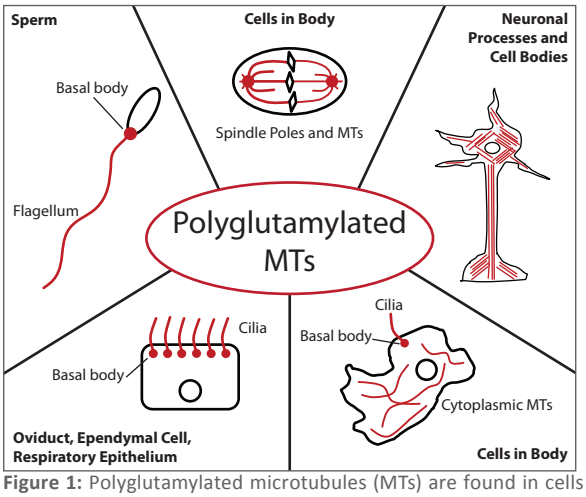

Figure 1: Polyglutamylated microtubules (MTs) are found in cells from protists to mammals. MTs are depicted in red.

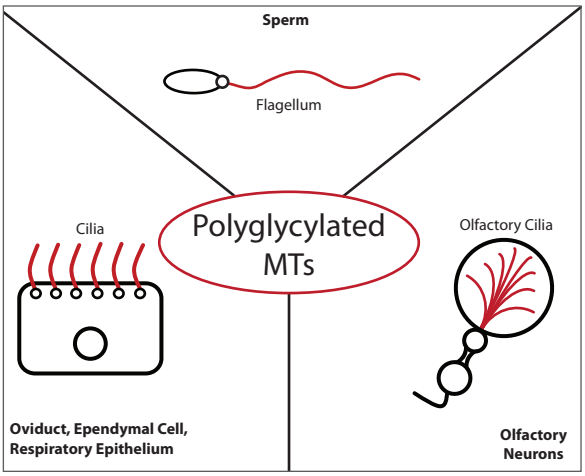

Figure 2: Polyglycylated microtubules (MTs) are found in cilia and flagella of cells from protists to mammals. MTs are depicted in red.

Until recently, one of the mysteries associated with tubulin polymodifications has been the identity of the glutamylation and glycylation enzymes. These enzymes are now known to be members of the tubulin tyrosine ligase-like (TTLL) family of proteins[7-11]. Specific TTLL proteins are involved with the initiation vs elongation steps of polymodification and exhibit a preference for the tubulin isoforms[7-11]. Both polymodifications form a variable number of peptide side chains that attach to the γ-carboxyl groups of glutamate residues in the C-terminal tails of α/β tubulin[7-11]. The C-terminal tail is where structural and motor MT-associated proteins (MAPs) bind[12], suggesting that PTMs could regulate such binding to confer MT functional diversity[1-3]. Indeed, recent *in vitro* studies demonstrate that tubulin polyglutamylation can modulate the binding of

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structural and motor MAPs to MTs, which could serve as a means of controlling the functional specificity of MT subpopulations[13-16].

Several other interesting studies have provided insights into the potential functions of polyglutamylation and polyglycylation. Polyglutamylation has been reported to promote MT severing *in vivo* and *in vitro*, suggesting that this PTM could act as a signal to control MT mass and stability[17]. Polyglutamylation could also affect neuron development as local MT severing is required for neurite outgrowth[17] and most neuronal MTs are highly polyglutamylated[2,4]. While an exact role for glutamylation and glycylation in ciliary or flagellar function is unknown, it is clear that these modifications are critical for normal ciliary function. Polyglutamylation of axonemal MTs of airway epithelial cilia is required for normal ciliary function involving dynein activity[18,20]. Likewise, polyglycylation is required for assembly and functioning of cilia and flagellar axonemes[7]. Indeed, RNAi knockdown of the TTLL3 glycylase in *Drosophila* testes caused abnormal sperm tail axonemes which correlated with decreased male viability and sterility[8]. Despite these recent gains in understanding tubulin polymodifications, much remains to be discovered, including the identity of all the tubulin deglutamylases and deglycylases[21-23].

Tubulin Research Tools

Unlabeled Proteins	Source	Purity	Cat. #	Amount
Tubulin Protein Lyophilized (no glycerol)	Porcine Brain	>99%	T240-A T240-B T240-C T240-DX	1 x 1 mg 5 x 1 mg 20 x 1 mg 1 x 10 mg
Tubulin Protein, MAP rich	Porcine Brain	70% tubulin 30% MAPs	ML116-A ML116-B ML116-C ML116-DX	1 x 1 mg 5 x 1 mg 20 x 1 mg 1 x 10 mg
Tubulin Protein Lyophilized	Bovine Brain	>99%	TL238-A TL238-B TL238-C TL238-D TL238-DX TL238-E	4 x 250 µg 1 x 1 mg 5 x 1 mg 10 x 1 mg 1 x 10 mg 20 x 1 mg
Tubulin for HTS Applications	Porcine Brain	97%	HTS03-A HTS03-B HTS03-XL	1 x 4 mg 1 x 40 mg 1 x 100 mg
Tubulin for HTS Applications	Bovine Brain	97%	HTS02-A HTS02-B HTS02-XL	1 x 4 mg 1 x 40 mg 1 x 100 mg
Tubulin Protein Frozen (no glycerol)	Porcine Brain	>99%	T238P-A T238P-B T238P-C	1 x 1 mg 5 x 1 mg 20 x 1 mg

Assays	Cat. #	Amount
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
Microtubule Binding Protein Spin-Down Assay Biochem Kit™	BK029	30-100 assays

Antibody	Cat. #	Amount
Tubulin polyclonal antibody (host: sheep) **Detects all species and isoforms of tubulin**	ATN02-A ATN02-B	1 x 100 µg 3 x 100 µg

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