

The Tubulin Code: *Post-translational Modifications of Tubulins*

The internal organization, shape, motility and life cycle of eukaryotic cells are all controlled by a complex network of polymeric filaments called the **cytoskeleton**, which includes actin filaments, intermediate filaments and microtubules. These polymers have important roles in arranging and maintaining the integrity of intracellular compartments. **Microtubules (MT)** are the largest cytoskeletal components involved in intracellular transport (cell signaling), cell migration/trafficking, cell division and proliferation. Microtubules control differentiative processes involving intracellular rearrangements and changes in morphology. Complex microtubule structures form the core components of centrosomes and the centrioles important for mitosis, and the core structures of cilia and flagella, which are called axonemes. Despite their functional diversity, all microtubules are assembled from heterodimers of **α -tubulin** and **β -tubulin**. Soluble α -tubulin- β -tubulin dimers polymerize into polar microtubules in the presence of GTP. Understanding of the cell structure and function is essential for gaining deeper knowledge of normal pathways such as morphogenesis, wound healing, neurogenesis and immune response, as well as abnormal processes such as metastasis and tumor-related angiogenesis.

In **neurons**, microtubules, actin filaments and neurofilaments compose the cytoskeleton, maintaining cell polarity, architecture and morphology. Regulation of microtubules polymerization is controlled by microtubule associated proteins, post-translational modifications of tubulin α and β , microtubules and signaling molecules. Deregulation of the neuronal cytoskeleton/MT function constitutes a key insult during the pathogenesis of nervous system diseases, leading to neurodegenerative diseases, including Amyotrophic Lateral Sclerosis, Alzheimer's Disease (AD), Hereditary Spastic Paraplegia, Parkinson's disease (PD) and others. **Posttranslational modifications (PTMs)** are highly dynamic and often reversible processes where protein functional properties are altered by addition of a chemical group or another protein to its amino acid residues. Tubulins and microtubules are major substrates for PTMs. PTMs include tyrosination/detyrosination, D2-tubulin formation, acetylation, phosphorylation, polyamination, ubiquitination, polyglutamylation and glycylation. PTMs are involved in fine-tuning of interactions between microtubules and different MT-interacting proteins.

LIT: Post-translational regulation of the microtubule cytoskeleton: mechanisms and functions: C. Janke & J.C. Bulinski; Nat. Rev. Mol. Cell Biol. **12**, 773 (2011) • The tubulin code: molecular components, readout mechanisms, and functions: C. Janke; J. Cell. Biol. **206**, 461 (2014) • Microtubules in health and degenerative disease of the nervous system: A.J. Matamoros & P.W. Baas; Brain. Res. Bull. **126**, 217 (2016) • The emerging role of the tubulin code: From the tubulin molecule to neuronal function and disease: S.Chakraborti, et al.; Cytoskeleton **73**, 521 (2016)

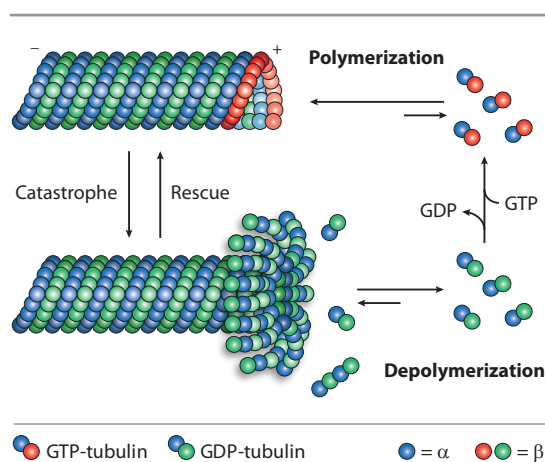


FIGURE: Microtubule dynamic instability. Polymerizing and rapidly depolymerizing polymers coexist at steady state.

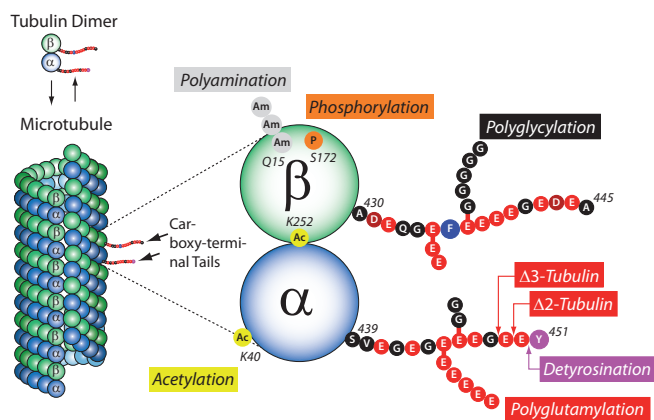


FIGURE 2: Tubulin PTM Overview. Adapted from C. Janke; J. Cell. Biol. **206**, 461 (2014).

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Validated Post-translational Modification-specific Antibodies

ANTIBODIES	PID	PRODUCT DESCRIPTION
anti-α-Tubulin (acetylated), mAb (TEU318)	AG-20B-0068	Detects K40 acetylation of α -tubulin; signal is specifically increased by modification with tubulin acetyl transferase α -TAT1. Works in Immunocytochemistry and Western blot.
anti-Polyglutamylation Modification, mAb (GT335)	AG-20B-0020	Recognizes the posttranslational modification (poly)glutamylation on proteins. Recognizes most forms of polyglutamylated tubulin (α - and β -tubulin), independent of the length of the glutamate side chains. Detects also other (poly)glutamylated proteins. The detection is not sequence-specific. However, an acidic environment of the modification site is required. Works in Immunocytochemistry, Immunohistochemistry, Immunoprecipitation and Western blot.
anti-Polyglutamate chain (polyE), pAb (IN105)	AG-25B-0030	Recognizes C-terminally located linear alpha-glutamate chains of 4 and more glutamate residues. Works in Immunocytochemistry, Immunohistochemistry and Western blot applications.
anti-Tubulin (glycylated), pAb (Gly-pep1)	AG-25B-0034	This antibody recognizes mono or bi-glycylated tubulins. The activity of glycylating enzymes (TTL3 and TTL8) in cultured cells leads mainly to the modification of α - and β -tubulin, but also of other, yet unidentified protein substrates also detected by the antibody Gly-pep1. In immunofluorescence labeling, the antibody strongly labels glycylated microtubules. As glycylation of microtubules is particularly found in cilia and flagella, Gly-pep1 labels motile cilia as well as some (mostly very long) primary cilia. Works in Immunocytochemistry, Immunoprecipitation and Western blot applications.

Recombinant Microtubule-target Antibodies

ANTIBODIES	PID	PRODUCT DESCRIPTION
anti-Tubulin-GTP, mAb (rec.) (MB11) UNIQUE	AG-27B-0009	Recognizes human, mouse, rat and drosophila tubulin-GTP in Immunocytochemistry
anti-α-Tubulin, mAb (rec.) (F2C)	AG-27B-0005	Recognizes mouse, bovine and human α -tubulin in Immunocytochemistry and Western blot.
anti-β-Tubulin, mAb (rec.) (S11B)	AG-27B-0008	Recognizes human, mouse, rat, pig, drosophila and monkey β -tubulin in Immunocytochemistry and Western blot.

Rab1-GTP and Rab6-GTP Specific Antibodies

Rab proteins, members of the small GTPase superfamily, are important regulators of vesicle transport via interactions with effector proteins and motor proteins. Rab1 and 6 are implicated in anterograde and retrograde trafficking in the secretory pathway. Recently, Rab1 has been shown to be involved in **autophagy** by helping the formation of the pre-autophagosomal isolation membrane (phagophore). Rab6 also functions as modulator of the unfolded protein response (UPR), helping the recovery from an ER stress insult. Rab6 is upregulated in Alzheimer's Disease.

ANTIBODIES	PID	PRODUCT DESCRIPTION
anti-Rab1-GTP, mAb (rec.) (ROF7)	AG-27B-0006	Recognizes human, mouse, rat and dog Rab1a-GTP and Rab1b-GTP in Immunocytochemistry and Immunoprecipitation.
anti-Rab6-GTP, mAb (rec.) (AA2)	AG-27B-0004	Recognizes human, mouse and Drosophila GTP-bound Rab6a and Rab6b and mutant Rab6Q72L. Does not detect Rab6-GDP. Works in Immunocytochemistry.
anti-Rab6-GTP, mAb (rec.) (AA2) (ATTO 488)	AG-27B-0004TD	Recognizes human, mouse and Drosophila GTP-bound Rab6a and Rab6b and mutant Rab6Q72L. Does not detect Rab6-GDP. Works in Immunocytochemistry.