[Cell Cycle 6:17, 2152-2160, 1 September 2007]; ©2007 Landes Bioscience

Review

The Tubulin Code

Kristen J. Verhey[1,*]
Jacek Gaertig[2]

[1]Department of Cell and Developmental Biology; University of Michigan Medical School; Ann Arbor, Michigan USA

[2]Department of Cellular Biology; University of Georgia; Athens, Georgia USA

*Correspondence to: Kristen J. Verhey; Department of Cell and Developmental Biology, University of Michigan Medical School; Ann Arbor, Michigan 48109 USA; Email kjverhey@umich.edu.

Original manuscript submitted: 06/25/07
Manuscript accepted: 06/26/07

Previously published online as a *Cell Cycle* E-publication:
http://www.landesbioscience.com/journals/cc/article/4633

KEY WORDS

microtubule, tubulin, posttranslational modification, acetylation, polyglutamylation, histone code

ABBREVIATIONS

PTM	posttranslational modification
CTT	C-terminal tail
TTL	tubulin tyrosine ligase
TTLL	TTL-like enzyme
MAP	microtubule associated protein
+TIP	plus-end tracking protein
CLIP	cytoplasmic linker protein

ACKNOWLEDGEMENTS

We thank Marie-Helene Bré, David Allis, and Ray Trievel for stimulating discussions and reading the manuscript. Work in our labs is supported by the NSF (033965 to J.G.) and NIH (GM070862 to K.J.V.).

ABSTRACT

Microtubules create diverse arrays with specific cellular functions such as the mitotic spindle, cilia and bundles inside neurons. How microtubules are regulated to enable specific functions is not well understood. Recent work has shown that posttranslational modifications of the tubulin building blocks mark subpopulations of microtubules and selectively affect downstream microtubule-based functions. In this way, the tubulin modifications generate a "code" that can be read by microtubule-associated proteins in a manner analogous to how the histone code directs diverse chromatin functions. Here we review recent progress in understanding how the tubulin code is generated, maintained, and read by microtubule effectors.

INTRODUCTION

Microtubules are cytoskeletal filaments that play important roles in diverse cellular functions including structural support, localization of organelles, segregation of chromosomes and intracellular trafficking. Microtubules are polymers of α/β-tubulin heterodimers that associate head-to-tail and laterally to form hollow tubes (Fig. 1). Microtubules can be organized into microtubule-based organelles with specialized functions, including the radial cytoplasmic network, cilia, centrioles and the mitotic/meiotic spindle. Singlet microtubules are the most ubiquitous form of the polymer, however, microtubules can be fused laterally into doublets (in cilia) or triplets (in centrioles and basal bodies).

Singlet microtubules are usually highly dynamic and undergo rapid turnover by exchange of subunits. The prevalent form of this turnover is known as dynamic instability where the ends of microtubules undergo rapid transitions between growth and shrinkage. Dynamic instability has been postulated to provide a space-probing mechanism critical for establishment of contacts between the ends of microtubules and target organelles (such as chromosomes during mitosis).[1] However, within the cytoplasmic network, there also exists a stable subpopulation of microtubules ($t_{1/2}$ = 1–2 hr for stable microtubules versus $t_{1/2}$ = 5–15 min for dynamic microtubules).[2-4] The cellular function of stable microtubules is unknown but it has been suggested that these microtubules are required for cellular morphogenesis.[5] A distinguishing feature of stable microtubules is that they acquire a variety of posttranslational modifications (PTMs) on tubulin dimers in a time-dependent manner. The microtubule doublets of cilia and triplets of centrioles are also very stable and highly enriched in PTMs. The functions of the evolutionarily-conserved microtubule PTMs are poorly understood. Recent studies from multiple laboratories, including our own, have led to a hypothesis that tubulin PTMs dictate the recruitment of protein complexes (microtubule effectors), which in turn contribute to microtubule-based functions in specific cellular locations. Thus, PTMs could be creating a "tubulin code" that in many ways is analogous to the "histone code" that has been proposed to regulate chromatin assembly and gene transcription.[6]

A TUBULIN CODE

Microtubules can acquire a variety of evolutionarily conserved PTMs including polyglutamylation, polyglycylation, detyrosination (and related Δ2 modification), acetylation, phosphorylation and palmitoylation (Table 1 and ref. 7). In most cases, the modification enzymes act preferentially on tubulin subunits already incorporated into microtubules. One exception is the recently discovered phosphorylation of β-tubulin on Ser172 that

©2007 LANDES BIOSCIENCE. DO NOT DISTRIBUTE.

Table 1 **Tubulin PTMs**

PTM	Description	Site(s)	Forward Enzyme(s)	Reverse Enzymes(s)
Detyrosination	removal of C-terminal tyrosine	terminal tyrosine on CTT of α-tubulin	Carboxypeptidase[31] (Nna1/CCP1?)[32]	TTL[33]
Glutamylation	addition of one or more glutamates as a side chain	multiple glutamates in the primary sequence of CTTs of α- and β- tubulin	TTLL1, TTLL5, TTLL6 (α-tubulin)[34,54] TTLL4, TtTTLL6Ap, TTLL7 (β-tubulin)[34,54,69]	unknown
Glycylation	addition of one or more glycines as a side chain	multiple glutamates in the primary sequence of CTTs of α- and β-tubulin	unknown	unknown
Acetylation	addition of acetyl group	Lys40 of α-tubulin	unknown	HDAC6, SirT2[39-41]
Phosphorylation	addition of phosphate	Ser172 and unknown site(s) on CTT of β-tubulin unknown sites on α-tubulin	Cdk1/cyclin B (Ser172 of β-tubulin)[8] PSK[59] Fes[60] Syk[57,58]	unknown
Palmitoylation	addition of palmitate lipid group	Cys376 of α-tubulin	unknown	unknown
Δ2	removal of penultimate glutamate from detyrosinated α-tubulin	CTT of α-tubulin	unknown	unknown

occurs on unpolymerized tubulin in mitotic cells and inhibits incorporation of heterodimers into the polymer.[8] Most PTMs are enriched on microtubules that are "stable" as defined by their slow subunit turnover and resistance to drugs that depolymerize microtubules such as nocodazole.[9-15] However, in vitro studies on purified tubulin have failed to detect any effect of acetylation or detyrosination on the polymer dynamics.[16-18] Thus, at least some PTMs do not affect polymer dynamics by changing the intrinsic properties of microtubules. Rather, an emerging hypothesis is that tubulin modifications specify a code that dictates biological outcomes through changes in higher-order microtubule structure and/or by recruiting and interacting with effector proteins. This hypothesis is analogous to the histone code hypothesis - that modifications on core histones, acting in a combinatorial or sequential fashion, specify multiple functions of chromatin such as changes in higher-order chromatin structure or selective activation of transcription.[19-21] The apparent parallels between these two types of structural frameworks, chromatin in the nucleus and microtubules in the cytoplasm, are intriguing and suggest that a general theme has evolved that regulates the functions of cellular polymers (Fig. 1).

One apparent parallel is that specific polymer regions can be distinguished biochemically and functionally by the presence of PTMs on their building blocks. Chromatin of genes active in transcription has increased acetylation on certain lysine residues of core histones.[20,22] In a similar fashion, PTMs on tubulin are enriched in restricted subcellular areas and therefore have the potential to locally adapt microtubules for specific functions. For example, microtubules oriented towards a wound in a confluent monolayer of cells are enriched in detyrosination and acetylation[23,24] and central spindle but not astral microtubules are marked by detyrosination, glutamylation and acetylation.[25-27] A second parallel between chromatin and microtubules is that most PTMs take place on the tail domains of histones and tubulins that comprise the outward face of the polymer (Fig. 1). In the case of α- and β-tubulin, most PTMs occur on the C-terminal tails (CTTs), essential domains[28] that could not be resolved in atomic models[29] but are known to comprise the binding region for a large number of microtubule binding proteins.[30] In the paragraphs below, we will review recent work supporting the existence of a "tubulin code" and discuss potential ramifications.

WHAT ARE THE ENZYMES THAT ESTABLISH THE TUBULIN CODE?

The discovery of the enzymes that deposit the modifications has long lagged behind the discovery of the modifications themselves. However, the last few years have been a time of rapid progress in the identification of microtubule PTM enzymes. Detyrosination involves the enzymatic removal of the C-terminal tyrosine of α-tubulin by a carboxypeptidase.[31] The identity of the tubulin carboxypeptidase has not been established despite multiple purification efforts. However, a recent study identified a novel cytosolic carboxypeptidase, Nna1/CCP1, that is abundant in tissues with high content of tubulin such as testis, pituitary and brain.[32] Mice lacking Nna1/CCP1 lack detectable detyrosinated α-tubulin in mitral cells of the olfactory bulb and experience degeneration of Purkinje cells and altered gait which indicates that detyrosination could be important. Nna1/CCP1 belong to a family of six related genes with some showing restricted pattern of expression.[32] Future biochemical studies should establish whether Nna1/CCP1 is the long-sought tubulin carboxypeptidase. The enzyme that carries out the reverse reaction and converts soluble α-tubulin back to its unmodified form, tubulin tyrosine ligase (TTL), was identified much earlier.[33] Interestingly, it appears that only mammals and trypanosomes have a TTL sequence in their genomes,[34] while detyrosination is widespread among eukaryotes.

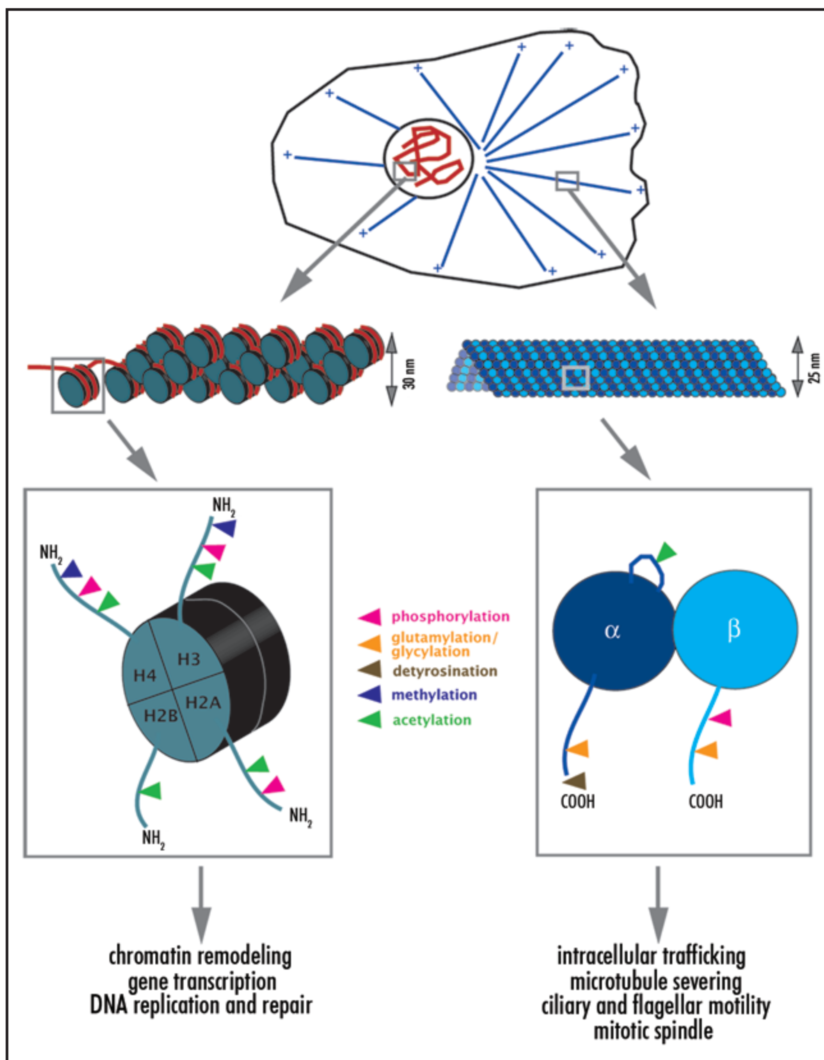

Figure 1. Parallels between the histone and tubulin codes in eukaryotic cells. In the nucleus, DNA (red) is organized into chromatin by winding around an octamer of core histones (two each of histones H2A, H2B, H3 and H4). The resulting nucleosomes are folded into a fiber about 30 nm in diameter, and these fibers can be further folded into higher-order structures (not shown). In the cytoplasm of an interphase cell, microtubules (blue) are polymerized from heterodimers of α/β-tubulin. The plus (fast-growing) ends of the microtubules extend out to the cell cortex. In both cases, portions of the polymer can be marked by PTMs of the histone or tubulin building blocks. Most of these PTMs occur on N- or C-terminal tail domains that are accessible on the polymer surface. Furthermore, in both cases multiple distinct PTM types can occur on the same tails creating combinatorial "PTM cassette" marks. These PTMs likely control specific biological functions by controlling the structure of the polymer and/or by recruiting specific protein complexes to the polymer.

Thus, either this PTM is irreversible in most eukaryotes or another enzyme (possibly a TTL-like protein, see below) exists that can restore tyrosine at the end of α-tubulin.

Acetylation of α-tubulin occurs on residue Lys-40[35] within a loop that is disordered in the crystal structure but thought to be located inside the microtubule lumen.[29] If acetylation occurs in the microtubule lumen, then acetylation and deacetylation enzymes must be capable of accessing the space inside the microtubule. It is relevant that recent studies identified particles inside the lumen of frozen singlet and doublet microtubules.[36-38] The enzyme that carries out acetylation of α-tubulin on microtubules has not been identified although two enzymes have been shown to carry out the reverse reaction in vitro and in vivo—HDAC6, a protein with sequence homology to histone deacetylases, and SIRT2, an enzyme that also plays a role in transcriptional silencing in yeast.[39-41]

Polyglycylation and polyglutamylation are polymeric modifications (polymodifications) that involve the attachment of polypeptide side chains made of glycines and glutamates, respectively, to specific glutamate residues in the CTT of both α- and β-tubulin.[42,43] Both of these modifications are relatively rare although non-tubulin targets have recently been identified.[44-46] In mammals, tubulin glycylation is mostly restricted to axonemes of motile cilia and flagella[47-49] whereas glutamylation is abundant in neurons, on centrioles, in axonemes, and in spindle microtubules.[27,50,51] In ciliated protists, both polymodifications are found in numerous microtubular networks, including cytoplasmic and axonemal microtubules.[47,52,53] A major breakthrough in the identification of PTM enzymes was achieved recently with the identification of a gene family that carries out tubulin glutamylation.[34] The glutamylases belong to the large family of TTL-like enzymes (TTLLs) as their catalytic region contains a domain homologous to TTL. The structural similarity between glutamylases and TTL reflects a common property of these two types of enzymes: they catalyze the addition of an amino acid to a glutamate residue in the tubulin CTT. While TTL ligates tyrosine to the exposed C-terminal glutamate residue via a standard peptide bond,[33] the TTLL polyglutamylase enzymes can catalyze two different reactions: first, the initiating glutamylation in which a glutamate residue is added to the γ-carboxyl group of the acceptor glutamate and second, the elongating glutamylation in which additional glutamates are added via an isopeptide bond. There appears to be a division of function in the ability of specific TTLL enzymes to initiate or elongate the glutamyl side chains. Thus, some TTLL glutamylases show a predominant chain initiating activity while other enzymes showed strong chain elongating activity.[54] Subtypes of TTLL glutamylases also differ in their preference for either α- or β-tubulin as a substrate. For example, the murine TTLL1 enzyme and its Tetrahymena ortholog, Ttll1p, prefer α-tubulin while Ttll6Ap of Tetrahymena prefers β-tubulin.[34] Finally, the enzymes that catalyze tubulin glycylation have not been determined but could be members of the TTLL family whose enzymatic properties have not yet been studied.

Phosphorylation of a serine residue in the CTT of β-tubulin in microtubules has been reported although the enzyme(s) responsible have not been identified.[55,56] In B lymphocytes, a tyrosine residue in the CTT of α-tubulin can be phosphorylated in vivo and in vitro by Syk, a non-receptor tyrosine kinase required for B- cell differentiation.[57,58] Outside of the CTT region, β-tubulin can be phosphorylated at Ser172 by Cdk1/cyclin B complex that regulates entry into mitosis.[8] Several other kinases have been shown to phosphorylate tubulin in vitro—prostate-derived sterile 20-like kinase (PSK)[59] and Fes protein tyrosine kinase[60]—but the in vivo relevance and modification sites are unknown.

HOW ARE PATTERNS OF MICROTUBULE PTMs ESTABLISHED?

One major difference between the histone and tubulin codes may be in the way the information is propagated between generations of organelles. There is considerable evidence that the histone code can be inherited and maintained by copying the pattern from preexisting chromatin onto newly assembled chromatin at the time of DNA replication.[61] The mechanism of this epigenetic transmission is likely based on the partitioning of preexisting histone particles to both strands of DNA during replication. Some microtubule-based organelles (e.g., centrosomes and basal bodies) are inherited by a template-driven mechanism where new structures are formed in the vicinity of preexisting structures.[62] However there is no evidence that the template organelle directly influences the PTM pattern in the newly formed organelle. Rather, the PTM pattern is recreated in the newly formed organelle in a gradual manner. For example, newly formed basal bodies and associated cilia have an immature pattern of PTMs characterized by shorter side chains of polyglycylation.[49,63] Thus, the state of PTM distinguishes between old and new microtubule structures and could target assembly factors to forming organelles. Other microtubule-based structures, such as cytoplasmic microtubules, the mitotic spindle and cilia, are formed de novo mostly, if not entirely, from unmodified tubulin heterodimers. Thus, in case of both template-dependent and -independent microtubular structures, PTM patterns are probably recreated without a direct influence of preexisting PTMs. How then specific patterns of tubulin PTMs are established is unknown but three major regulatory mechanisms can be envisioned.

One attractive mechanism of control involves spatial and temporal regulation of the activity of the PTM enzymes (both forward and reverse). For example, in wounded cell models, plasma membrane-associated members of the Rho, Rac and Cdc42 GTPase families trigger localized changes in both actin and microtubule dynamics that lead to cell polarization and directed motility (reviewed in refs. 64–66). Importantly, downstream effectors of these GTPases include the microtubule plus-end tracking proteins (+TIPs) that "capture" and "stabilize" the ends of microtubules oriented towards the leading edge of the cell. Recent work has shown that activated versions of the +TIP proteins EB1, APC and CLASP can stimulate the formation of both detyrosinated and acetylated microtubules in wounded fibroblasts.[24,67] Yet whether GTPases and +TIP proteins directly impinge on the PTM enzymes has not been tested.

A second possible mechanism of regulation of PTM enzymes involves their subcellular localization. PGs1, a noncatalytic subunit of the TTLL1 α-tubulin glutamylase complex, localizes preferentially to major sites of tubulin glutamylation, notably centrosomes and basal bodies, axonemes, and the distal portion of neurites. Interestingly, this localization is regulated during the cell cycle as PGs1 localization is predominantly cytosolic during mitosis.[68] In cultured neurons, the β-tubulin-preferring glutamylase TTLL7 is enriched in the somatodendritic regions and this localization correlates with the higher levels of glutamylation on β-tubulin in dendrites as compared to axons.[69] The Ttll6Ap β-tubulin polyglutamylase specifically localizes to motile cilia in Tetrahymena[34] and similar glutamylases localize to nonmotile sensory (primary) cilia in mammalian cells.[54] Interestingly, there are striking differences in the pattern of PTMs inside the cilium. For example, in doublet microtubules, detyrosination and both polymodifications occur mainly on the B-tubule while the A-tubule is largely unmodified.[70-73] This could reflect the ability of modifying enzymes to associate with only a subset of microtubules and at specific positions within the lattice.

A third possibility is that the microtubule substrate is regulated in a way that controls their access or exposure time to PTM enzymes. In one scenario, the modification of subsets of microtubules is simply a time-dependent phenomenon, that is, microtubules that are "stabilized" remain in place long enough for the PTM enzymes to work. An alternative mechanism is that "stabilized" microtubules exist in an unknown structural state that makes them the preferred substrate for PTM addition. Indirect support for this possibility comes from the fact that pharmacological treatments that stabilize microtubules (e.g., taxol) result in increased levels of several PTMs including detyrosination, acetylation and glycylation (refs. 26, 74 and Rogowski K, Gaertig J, unpublished). It should also be considered that PTM patterns could be regulated by competition between PTM enzymes and other proteins that bind to similar sites on the microtubule polymer. Elucidation of the molecular mechanisms by which microtubule stability and the PTM enzymes are controlled, so far hindered by the lack of identification of the enzymes, will provide fertile ground for future work.

WHO ARE THE INTERPRETERS OF THE TUBULIN CODE?

A major implication of the tubulin code is that PTMs influence the recruitment of protein complexes (microtubule effectors), which in turn contribute to microtubule-based functions. Three major classes of microtubule binding proteins can be considered as interpreters of the tubulin code. First, microtubule associated proteins (MAPs) such as Tau, MAP1 and MAP2 that bind statically along the length of microtubules. Second, plus end tracking proteins (+TIPs) that bind in a transient manner to the plus-ends of growing microtubules. And third, molecular motors that use the energy of ATP hydrolysis to carry cargoes along microtubule tracks.

MAPs. Functional roles of structural MAPs are not completely understood but are thought to contribute to the stability and organization of microtubules, especially in neuronal cells.[75] In vitro, Tau, MAP1B, and MAP2 bind preferentially to tubulins with moderate levels of polyglutamylation (~3 glutamyl units) whereas MAP1A shows optimal affinity for highly modified tubulins (~6 glutamyl units).[76-78] As α-tubulin glutamylation is abundant in very young neurons whereas β-tubulin glutamylation increases during post-natal development,[50] glutamylation could control transitions in MAP binding during neuronal development.[78] Lys 40 α-tubulin acetylation may also influence MAP binding as overexpression of HDAC6 delocalized p58, a MAP involved in the association of Golgi membranes with microtubules.[79]

+TIPs. Recent work has shown that tubulin detyrosination negatively affects the association of some +TIPs with microtubules. In yeast, removal of the C-terminal aromatic residue (phenylalanine) of α-tubulin disabled the interaction of Bik1p, a homolog of the mammalian cytoplasmic linker protein 170 (CLIP-170), with microtubule plus ends but had no effect on the association of Bim1p, the EB1 +TIP homolog.[80] While it is not known whether such PTM occurs naturally in yeast, this experiment showed that the state of the C-terminal amino acid on α-tubulin has profound consequences in vivo. These results led to the hypothesis that the presence of unmodified α-tubulin at microtubule plus-ends plays an important role in localization of members of the CLIP-170 family of +TIP proteins. Indeed, in neurons and fibroblasts isolated from TTL-null mice, increased levels of detyrosination resulted in mislocalization of CLIP-170 and p150Glued whereas other +TIP proteins such as EB1 were unaffected.[81,82] CLIP-170 and p150Glued both have

a CAP-Gly domain. Structural work has shown that the CAP-Gly domain has a binding groove that may directly recognize the unmodified C-terminal sequence of α-tubulin.[83] Taken together, these results indicate that +TIP proteins containing a CAP-Gly microtubule-binding domain require the presence of tyrosinated α-tubulin for their preferential localization to microtubule plus ends.

Motors. Studies in a wide variety of cell types have shown that cargoes delivered by motors can be targeted to specific subcellular destinations, such as cilia,[84] axons or dendrites,[85,86] and the leading edge of migrating fibroblasts.[87] Furthermore, cargoes can even be targeted to subsets of microtubules within the mitotic spindle, the axon, and ciliary axoneme.[88-90] Thus, the idea that microtubule PTMs could serve as "road signs" to direct motor transport to specific subcellular destinations has long been an attractive one. Early studies showed that the addition of antibodies that specifically recognize detyrosinated tubulin prevented binding of Kinesin-1 to microtubules in vitro whereas antibodies to tyrosinated tubulin had no effect.[91,92] Gel overlay and antibody inhibition experiments have shown that Kinesin-1 also binds preferentially to tubulin containing 3 glutamyl units.[77] To directly examine the influence of PTMs on motors, recent experiments have utilized microtubules lacking specific PTMs due to genetic ablation of either the PTM sites or enzymes. Reed et al showed that loss of α-tubulin acetylation, α-tubulin detyrosination, or β-tubulin polymodifications resulted in decreased binding of Kinesin-1 to microtubules whereas loss of α-tubulin polymodifications had no effect.[86] Acetylation of α-tubulin also positively regulates cytoplasmic dynein binding to microtubules.[93] The effect of α-tubulin glutamylation has been examined using mice that lack functional PGs1, a noncatalytic subunit of TTLL1.[34,68,94] The deficiency in α-tubulin glutamylation is associated with decreased binding of several motors to microtubules in vitro, however, the main effect in mutant ($PGs1^{-/-}$) brains and cells was on the subcellular distribution of the kinesin-3 motor Kif1A and its cargo synaptic vesicles.[95] The possibility that decreased α-tubulin tyrosination in $PGs1^{-/-}$ mice could affect motor binding and motility, either directly or indirectly, cannot be ruled out presently. Further work is needed to elucidate the molecular mechanisms by which tubulin PTMs influence motor-microtubule interactions and motility. In particular, structural approaches are required to determine how the presence of PTMs affects the conformation of the polymer lattice.

WHAT ARE THE BIOLOGICAL CONSEQUENCES OF THE TUBULIN CODE?

Intracellular trafficking. A role for tubulin modifications in directing intracellular trafficking was suggested early on based on microinjection of antibodies that recognize specific PTMs. Antibodies that specifically recognize detyrosinated tubulin inhibited two kinesin-dependent processes, the recycling of transferrin receptors to the plasma membrane and the extension of vimentin intermediate filaments.[91,96,97] An antibody that recognizes mono- and polyglutamylated tubulin (GT335) interfered with kinesin-2-based pigment granule dispersion but not dynein-based aggregation in melanophores.[98]

With the identification of the enzymes that carry out tubulin modifications, more recent studies have used pharmacological or genetic methods to eliminate or enhance specific PTMs. Mice lacking functional TTL die soon after birth due to disorganization of neuronal networks[81] and fibroblasts cultured from these mice show defects in cell morphology during interphase.[82] Mice that are null for PGs1, a noncatalytic subunit of TTLL1 α-tubulin polyglutamylase, show mislocalization of synaptic vesicles, impaired synaptic transmission[95] and disorganized axonemes of sperm flagella.[94] In cultured neuronal cells, siRNA-mediated knockdown of TTLL7, a β-tubulin polyglutamylase, resulted in decreased neurite outgrowth.[69]

Surprisingly, elimination of acetylation in Chlamydomonas or Tetrahymena has no obvious phenotypic consequences and expression of a non-acetylatable α-tubulin in *C. elegans* rescues defects in neurons lacking MEC-12, the only identified tubulin in this organism that contains lysine at position 40.[99-101] Thus, α-tubulin acetylation is not required for cell survival but recent work has demonstrated an important role for this PTM in differentiated cell types of vertebrates. Pharmacological inhibition of deacetylases results in hyperacetylation of microtubules that can affect a variety of intracellular trafficking events such as the selective transport of the Kinesin-1 cargo JIP1 to a subset of neurites,[86] anterograde and retrograde transport of brain-derived neurotrophic factor (BDNF)-containing vesicles,[93] dynein/dynactin transport of aggresomes,[102,103] the exocytosis of interleukin (IL)-1β-containing secretory lysosomes,[104] as well as cytoskeletal rearrangements at the immune synapse.[105] Several studies have implicated a role for microtubule acetylation in cell motility—overexpression of HDAC6 leads to decreased acetylation and increased cell motility whereas inhibition of HDAC6 results in increased acetylation and decreased motility.[39,79] One of the potential mechanisms by which HDAC6 contributes to cell motility was revealed in a recent report showing that HDAC6-inhibited migrating cells have decreased microtubule dynamics and decreased focal adhesion turnover.[106] Taken together, these studies have provided important new advances in support of a tubulin code that directs intracellular trafficking.

Assembly and motility of cilia. Polyglycylation is a conserved PTM that is abundant in cell types with cilia. In the ciliated protist Tetrahymena, polyglycylation appears to be essential based on experiments in which α- or β-tubulin genes were replaced by mutated versions that lack modification sites. While elimination of polyglycylation sites on α-tubulin had no effect, elimination of polyglycylation sites on the β-tubulin CTT was lethal. Strains with reduced levels of glycylation resulted in defects in axonemal structure, ciliary motility and cytokinesis, Strikingly, glycylation site-deficient mutants had specific defects in the axoneme, including defects in assembly of the central pair microtubules and in B-tubule assembly.[107-109] These studies indicate that tubulin glycylation plays an important role in assembly of axonemal microtubules. One limitation to these studies is that ciliary tubulins are also extensively polyglutamylated on their CTTs.[110] The respective roles of polyglutamylation and polyglycylation in the assembly of cilia need to be dissected and this task can now be attempted by direct manipulation of specific forward enzymes (TTLLs). Glutamylation and glycylation likely also play important roles in regulation of ciliary beating once the organelle is assembled as antibodies that recognize either polyglutamate or polyglycine side chains interfered with ciliary beating in ATP-reactivated axonemes.[47,111,112]

Microtubule dynamics. There is no evidence that tubulin PTMs affect the intrinsic properties of microtubules such as their dynamicity. Yet several lines of evidence indicate that tubulin modifications may affect microtubule dynamics in vivo, possibly by regulating effectors that are important for turnover of microtubules. First, glutamylation may be important for the structural stability of centrioles.[113] Second, some reports have indicated that inhibition of HDAC6 tubulin deacetylase led to increased microtubule

stability in vivo[40,106] although other studies have failed to detect such effects.[79,114] The differences between these studies could be related to the use of assays with different levels of sensitivity. Third, recent experiments have unraveled a relationship between microtubule PTMs and microtubule severing. Katanin and spastin are AAA type ATPases that regulate microtubule dynamics by severing microtubules.[115] Mutations in spastin are responsible for the most frequent form of hereditary spastic paraplegia, a human neurodegenerative disease.[116] Katanin and spastin require the CTT domains of tubulins for severing activity[115,117] and spastin strongly interacts with the CTT of α-tubulin.[118] Thus, it is possible that PTMs located on CTTs regulate the activity of severing factors. In support of this, mutation of a glutamate residue on the CTT of β-tubulin in *C.elegans* suppressed the lethal phenotype resulting from overexpression of the catalytic subunit of katanin.[119] In addition, the increased stability of cortical microtubules seen upon mutation of several adjacent glutamates that serve as acceptor sites for polymodifications in Tetrahymena can be phenocopied by a knockout of the katanin gene.[107,108,120] However, the relationship between PTMs and microtubule severing proteins could be mutual as mice with a mutation in spastin displayed axonal swellings with increased density of microtubules that were excessively detyrosinated. Interestingly, the swellings showed signs of impairment in retrograde (but not anterograde) axonal transport.[121] It is therefore possible that lack of spastin severing activity decreases the turnover of microtubules which in turn leads to excessive modifications on microtubules. Recent studies in *Drosophila* support this model as a restricted knockdown of spastin in the nervous system caused excessive acetylation of microtubules at the neuromuscular junction and affected synaptic activity. Remarkably, synaptic defects caused by decreased or increased spastin function could be partially reversed by exposure to pharmacological agents that destabilize or stabilize microtubules, respectively.[122,123] The simplest explanation for these data is that spastin activity promotes turnover of microtubules and indirectly decreases the levels of PTMs. Taken together, these studies indicate that a mutual interaction could exist between PTMs and microtubule severing factors. On one hand, microtubule severing factors could recognize preferentially modified microtubules. On the other hand, the severing activity could increase the turnover of microtubules that in turn negatively regulates all PTMs that accumulate on stable microtubules.

Mitosis. Several PTMs are present on spindle and midbody microtubules but are absent from astral microtubules.[25-27] Thus, tubulin PTMs may play a role in directing mitotic events including targeting of effector proteins to a subset of microtubules (for examples, see Refs. 124-128). In support of this, increased levels of detyrosinated tubulin seen in fibroblasts cultured from TTL null mice resulted in defects in spindle orientation[82] and yeast cells expressing only detyrosinated α-tubulin displayed defects in nuclear positioning and spindle dynamics.[80] In both animal and human cancers, TTL activity is often suppressed during tumor growth indicating that TTL suppression and resulting excessive tubulin detyrosination represent a strong selective advantage for proliferating transformed cells.[129,130] In studies on polyglutamylation, Eddé and colleagues showed that polyglutamylase activity peaks in G_2 whereas the levels of polyglutamylated tubulin peak during mitosis.[51] In addition, microinjection of antibody GT335 that recognizes mono-and polyglutamylated tubulins caused a transient disappearance of centrioles and spindle defects.[113] Palmitoylation occurs on Cys376 of vertebrate α-tubulin[131,132] and mutation of the corresponding Cys377 residue to serine in yeast affected aspects of mitosis that involve interactions of astral microtubules with the cell cortex such as translocation of spindles to the bud.[133] Thus, palmitoylation of astral microtubules could tether spindle microtubules to the plasma membrane.

CAN PTMs AFFECT EACH OTHER?

The close apposition of several of the PTMs on the α- and β-tubulin CTTs raises the possibility that distinct PTMs influence each other. One mechanism of influence is that individual PTMs could influence the rate or activity of other PTM enzymes that act on the same CTT ("cis-tail" effects). In the case of the glutamylation enzymes, initial glutamylation sets the stage for elongation enzymes.[54] In mice lacking PGs1, a noncatalytic subunit of the α-tubulin polyglutamylase TTLL1, a decrease in α-tubulin tyrosination was detected despite no change in the levels of α-tubulin acetylation or β-tubulin polyglutamylation.[95] Individual PTMs could also have "trans-tail" effects where modification on one CTT affects modifications on the neighboring CTTs of the same microtubule. Cross-talk appears to occur between α- and β-tubulin subunits as site-directed mutagenesis of the glutamates that serve as sites of polymodification in Tetrahymena α-tubulin affected the levels of polymodifications on the nonmutated β-tubulin subunit.[110] It remains to be established whether cross-talk between different PTMs is a result of direct effects on PTM enzymes or an indirect effect of changes in microtubule structure or dynamics.

A second mechanism by which distinct PTMs can influence each other is that, in analogy to the histone code,[134] the tubulin code has the potential for generation of a combinatorial readout. For example, multiple PTMs (polyglycylation, polyglutamylation and detyrosination) were found on the same CTTs of axonemal tubulins.[110] Thus, the activity a particular microtubule effector could depend on the presence of multiple PTMs on the same CTT. The use of multiple marks would amplify the readout causing greater changes in microtubule-based processes than individual modifications.

FUTURE DIRECTIONS

Recent years have been a time of exciting progress in the identification of the enzymes that carry out tubulin PTMs. Hints about how tubulin PTMs influence effector proteins, such as molecular motors, and cellular functions, such as intracellular trafficking, have also emerged recently. However, a great deal of work is still needed to identify the unknown forward and reverse enzymes (such as tubulin acetyltransferase, polyglycylase, deglycylase and deglutamylase), to determine how PTMs affect the structure of the microtubule lattice, and to elucidate the physiological functions for PTMs in diverse cell types. Together this work will be important for deciphering the tubulin code to understand how PTM of specific microtubule tracks influences the recruitment of protein complexes and regulates microtubule-based functions.

References

1. Kirschner MW, Mitchison T. Microtubule dynamics. Nature 1986; 324:621.
2. Saxton WM, Stemple DL, Leslie RJ, Salmon ED, Zavortink M, McIntosh JR. Tubulin dynamics in cultured mammalian cells. J Cell Biol 1984; 99:2175-86.
3. Schulze E, Kirschner M. Microtubule dynamics in interphase cells. J Cell Biol 1986; 102:1020-31.
4. Schulze E, Kirschner M. Dynamic and stable populations of microtubules in cells. J Cell Biol 1987; 104:277-88.
5. Kirschner M, Mitchison T. Beyond self-assembly: From microtubules to morphogenesis. Cell 1986; 45:329-42.
6. Strahl BD, Allis CD. The language of covalent histone modifications. Nature 2000; 403:41-5.

7. Westermann S, Weber K. Post-translational modifications regulate microtubule function. Nat Rev Mol Cell Biol 2003; 4:938-47.
8. Fourest-Lieuvin A, Peris L, Gache V, Garcia-Saez I, Juillan-Binard C, Lantez V, Job D. Microtubule regulation in mitosis: Tubulin phosphorylation by the cyclin-dependent kinase Cdk1. Mol Biol Cell 2006; 17:1041-50.
9. Bre MH, Kreis TE, Karsenti E. Control of microtubule nucleation and stability in Madin-Darby canine kidney cells: The occurrence of noncentrosomal, stable detyrosinated microtubules. J Cell Biol 1987; 105:1283-96.
10. Schulze E, Asai DJ, Bulinski JC, Kirschner M. Posttranslational modification and microtubule stability. J Cell Biol 1987; 105:2167-77.
11. Khawaja S, Gundersen GG, Bulinski JC. Enhanced stability of microtubules enriched in detyrosinated tubulin is not a direct function of detyrosination level. J Cell Biol 1988; 106:141-9.
12. Webster DR, Borisy GG. Microtubules are acetylated in domains that turn over slowly. J Cell Sci 1989; 92:57-65.
13. Webster DR, Gundersen GG, Bulinski JC, Borisy GG. Differential turnover of tyrosinated and detyrosinated microtubules. Proc Natl Acad Sci USA 1987; 84:9040-4.
14. Webster DR, Gundersen GG, Bulinski JC, Borisy GG. Assembly and turnover of detyrosinated tubulin in vivo. J Cell Biol 1987; 105:265-76.
15. Bulinski JC, Gundersen GG. Stabilization of post-translational modification of microtubules during cellular morphogenesis. Bioessays 1991; 13:285-93.
16. Maruta H, Greer K, Rosenbaum JL. The acetylation of alpha-tubulin and its relationship to the assembly and disassembly of microtubules. J Cell Biol 1986; 103:571-9.
17. Paturle L, Wehland J, Margolis RL, Job D. Complete separation of tyrosinated, detyrosinated, and nontyrosinatable brain tubulin subpopulations using affinity chromatography. Biochemistry 1989; 28:2698-704.
18. Webster DR, Wehland J, Weber K, Borisy GG. Detyrosination of alpha tubulin does not stabilize microtubules in vivo. J Cell Biol 1990; 111:113-22.
19. Shahbazian MD, Grunstein M. Functions of site-specific histone acetylation and deacetylation. Annu Rev Biochem 2007; 76:75-100.
20. Kouzarides T. Chromatin modifications and their function. Cell 2007; 128:693-705.
21. Turner BM. Defining an epigenetic code. Nat Cell Biol 2007; 9:2-6.
22. Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell 2007; 128:707-19.
23. Gundersen GG, Bulinski JC. Selective stabilization of microtubules oriented toward the direction of cell migration. Proc Natl Acad Sci USA 1988; 85:5946-50.
24. Akhmanova A, Hoogenraad CC, Drabek K, Stepanova T, Dortland B, Verkerk T, Vermeulen W, Burgering BM, De Zeeuw CI, Grosveld F, Galjart N. Clasps are CLIP-115 and -170 associating proteins involved in the regional regulation of microtubule dynamics in motile fibroblasts. Cell 2001; 104:923-35.
25. Gundersen GG, Kalnoski MH, Bulinski JC. Distinct populations of microtubules: Tyrosinated and nontyrosinated alpha tubulin are distributed differently in vivo. Cell 1984; 38:779-89.
26. Piperno G, LeDizet M, Chang XJ. Microtubules containing acetylated alpha-tubulin in mammalian cells in culture. J Cell Biol 1987; 104:289-302.
27. Bobinnec Y, Moudjou M, Fouquet JP, Desbruyeres E, Edde B, Bornens M. Glutamylation of centriole and cytoplasmic tubulin in proliferating non-neuronal cells. Cell Motil Cytoskeleton 1998; 39:223-32.
28. Duan J, Gorovsky MA. Both carboxy-terminal tails of alpha- and beta-tubulin are essential, but either one will suffice. Curr Biol 2002; 12:313-6.
29. Nogales E, Wolf SG, Downing KH. Structure of the alpha beta tubulin dimer by electron crystallography. Nature 1998; 391:199-203.
30. Lakamper S, Meyhofer E. Back on track - On the role of the microtubule for kinesin motility and cellular function. J Muscle Res Cell Motil 2006; 27:161-71.
31. Hallak ME, Rodriguez JA, Barra HS, Caputto R. Release of tyrosine from tyrosinated tubulin: Some common factors that affect this process and the assembly of tubulin. FEBS Lett 1977; 73:147-50.
32. Kalinina E, Biswas R, Berezniuk I, Hermoso A, Aviles FX, Fricker LD. A novel subfamily of mouse cytosolic carboxypeptidases. Faseb J 2007; 21:836-50.
33. Ersfeld K, Wehland J, Plessmann U, Dodemont H, Gerke V, Weber K. Characterization of the tubulin-tyrosine ligase. J Cell Biol 1993; 120:725-32.
34. Janke C, Rogowski K, Wloga D, Regnard C, Kajava AV, Strub JM, Temurak N, van Dijk J, Boucher D, van Dorsselaer A, Suryavanshi S, Gaertig J, Edde B. Tubulin polyglutamylase enzymes are members of the TTL domain protein family. Science 2005; 308:1758-62.
35. L'Hernault SW, Rosenbaum J. Chlamydomonas alpha-tubulin is posttranslationally modified by acetylation on the epsilon-amino group of a lysine. Biochemistry 1985; 24:473-8.
36. Garvalov BK, Zuber B, Bouchet-Marquis C, Kudryashev M, Gruska M, Beck M, Leis A, Frischknecht F, Bradke F, Baumeister W, Dubochet J, Cyrklaff M. Luminal particles within cellular microtubules. J Cell Biol 2006; 174:759-65.
37. Nicastro D, Schwartz C, Pierson J, Gaudette R, Porter ME, McIntosh JR. The molecular architecture of axonemes revealed by cryoelectron tomography. Science 2006; 313:944-8.
38. Sui H, Downing KH. Molecular architecture of axonemal microtubule doublets revealed by cryo-electron tomography. Nature 2006; 442:475-8.
39. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP. HDAC6 is a microtubule-associated deacetylase. Nature 2002; 417:455-8.
40. Matsuyama A, Shimazu T, Sumida Y, Saito A, Yoshimatsu Y, Seigneurin-Berny D, Osada H, Komatsu Y, Nishino N, Khochbin S, Horinouchi S, Yoshida M. In vivo destabilization of dynamic microtubules by HDAC6-mediated deacetylation. EMBO J 2002; 21:6820-31.
41. North BJ, Marshall BL, Borra MT, Denu JM, Verdin E. The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. Mol Cell 2003; 11:437-44.
42. Redeker V, Levilliers N, Schmitter JM, Le Caer JP, Rossier J, Adoutte A, Bre MH. Polyglycylation of tubulin: A posttranslational modification in axonemal microtubules. Science 1994; 266:1688-91.
43. Edde B, Rossier J, Le Caer JP, Desbruyeres E, Gros F, Denoulet P. Posttranslational glutamylation of alpha-tubulin. Science 1990; 247:83-5.
44. Lalle M, Salzano AM, Crescenzi M, Pozio E. The Giardia duodenalis 14-3-3 protein is post-translationally modified by phosphorylation and polyglycylation of the C-terminal tail. J Biol Chem 2006; 281:5137-48.
45. Regnard C, Desbruyeres E, Huet JC, Beauvallet C, Pernollet JC, Edde B. Polyglutamylation of nucleosome assembly proteins. J Biol Chem 2000; 275:15969-76.
46. Xie R, Clark KM, Gorovsky MA. Endoplasmic reticulum retention signal-dependent glycylation of the Hsp70/Grp170-related Pgp1p in Tetrahymena. Eukaryot Cell 2007; 6:388-97.
47. Bre MH, Redeker V, Quibell M, Darmanaden-Delorme J, Bressac C, Cosson J, Huitorel P, Schmitter JM, Rossler J, Johnson T, Adoutte A, Levilliers N. Axonemal tubulin polyglycylation probed with two monoclonal antibodies: Widespread evolutionary distribution, appearance during spermatozoan maturation and possible function in motility. J Cell Sci 1996; 109:727-38.
48. Bressac C, Bre MH, Darmanaden-Delorme J, Laurent M, Levilliers N, Fleury A. A massive new posttranslational modification occurs on axonemal tubulin at the final step of spermatogenesis in *Drosophila*. Eur J Cell Biol 1995; 67:346-55.
49. Iftode F, Clerot JC, Levilliers N, Bre MH. Tubulin polyglycylation: A morphogenetic marker in ciliates. Biol Cell 2000; 92:615-28.
50. Audebert S, Koulakoff A, Berwald-Netter Y, Gros F, Denoulet P, Edde B. Developmental regulation of polyglutamylated alpha- and beta-tubulin in mouse brain neurons. J Cell Sci 1994; 107:2313-22.
51. Regnard C, Desbruyeres E, Denoulet P, Edde B. Tubulin polyglutamylase: Isozymic variants and regulation during the cell cycle in HeLa cells. J Cell Sci 1999; 112:4281-9.
52. Bre MH, de Nechaud B, Wolff A, Fleury A. Glutamylated tubulin probed in ciliates with the monoclonal antibody GT335. Cell Motil Cytoskeleton 1994; 27:337-49.
53. Bre MH, Redeker V, Vinh J, Rossier J, Levilliers N. Tubulin polyglycylation: Differential posttranslational modification of dynamic cytoplasmic and stable axonemal microtubules in paramecium. Mol Biol Cell 1998; 9:2655-65.
54. van Dijk J, Rogowski K, Miro J, Lacroix B, Edde B, Janke C. A targeted multienzyme mechanism for selective microtubule polyglutamylation. Mol Cell 2007; 26:437-48.
55. Gard DL, Kirschner MW. A polymer-dependent increase in phosphorylation of beta-tubulin accompanies differentiation of a mouse neuroblastoma cell line. J Cell Biol 1985; 100:764-74.
56. Pucciarelli S, Ballarini P, Miceli C. Cold-adapted microtubules: Characterization of tubulin posttranslational modifications in the Antarctic ciliate Euplotes focardii. Cell Motil Cytoskeleton 1997; 38:329-40.
57. Faruki S, Geahlen RL, Asai DJ. Syk-dependent phosphorylation of microtubules in activated B-lymphocytes. J Cell Sci 2000; 113:2557-65.
58. Peters JD, Furlong MT, Asai DJ, Harrison ML, Geahlen RL. Syk, activated by cross-linking the B-cell antigen receptor, localizes to the cytosol where it interacts with and phosphorylates alpha-tubulin on tyrosine. J Biol Chem 1996; 271:4755-62.
59. Mitsopoulos C, Zihni C, Garg R, Ridley AJ, Morris JDH. The Prostate-derived Sterile 20-like Kinase (PSK) regulates microtubule organization and stability. J Biol Chem 2003; 278:18085-0891.
60. Laurent CE, Delfino FJ, Cheng HY, Smithgall TE. The human c-fes tyrosine kinase binds tubulin and microtubules through separate domains and promotes microtubule assembly. Mol Cell Biol 2004; 24:9351-8.
61. Groth A, Rocha W, Verreault A, Almouzni G. Chromatin challenges during DNA replication and repair. Cell 2007; 128:721-33.
62. Bettencourt-Dias M, Glover DM. Centrosome biogenesis and function: Centrosomics brings new understanding. Nat Rev Mol Cell Biol 2007; 8:451-63.
63. Brown JM, Marsala C, Kosoy R, Gaertig J. Kinesin-II is preferentially targeted to assembling cilia and is required for ciliogenesis and normal cytokinesis in Tetrahymena. Mol Biol Cell 1999; 10:3081-96.
64. Wittmann T, Waterman-Storer CM. Cell motility: Can Rho GTPases and microtubules point the way? J Cell Sci 2001; 114:3795-803.
65. Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR. Cell migration: Integrating signals from front to back. Science 2003; 302:1704-9.
66. Watanabe T, Noritake J, Kaibuchi K. Regulation of microtubules in cell migration. Trends Cell Biol 2005; 15:76-83.
67. Wen Y, Eng CH, Schmoranzer J, Cabrera-Poch N, Morris EJ, Chen M, Wallar BJ, Alberts AS, Gundersen GG. EB1 and APC bind to mDia to stabilize microtubules downstream of Rho and promote cell migration. Nat Cell Biol 2004; 6:820-30.
68. Regnard C, Fesquet D, Janke C, Boucher D, Desbruyeres E, Koulakoff A, Insina C, Travo P, Edde B. Characterisation of PGs1, a subunit of a protein complex co-purifying with tubulin polyglutamylase. J Cell Sci 2003; 116:4181-90.
69. Ikegami K, Mukai M, Tsuchida J, Heier RL, Macgregor GR, Setou M. TTLL7 is a mammalian beta-tubulin polyglutamylase required for growth of MAP2-positive neurites. J Biol Chem 2006; 281:30707-16.

70. Fouquet JP, Prigent Y, Kann ML. Compartive immunogold analysis of tubulin isoforms in the mouse sperm flagellum: Unique distribution of glutamylated tubulin. Mol Reprod Dev 1996; 43:358-65.
71. Johnson KA. The axonemal microtubules of the Chlamydomonas flagellum differ in tubulin isoform content. J Cell Sci 1998; 111:313-20.
72. Multigner L, Pignot-Paintrand I, Saoudi Y, Job D, Plassmann U, Rudiger M, Weber K. The A and B tubules of the outer doublets of sea urchin sperm axonemes are composed of different tubulin variants. Biochemistry 1996; 35:10862-71.
73. Kann ML, Prigent Y, Levilliers N, Bre MH, Fouquet JP. Expression of glycylated tubulin during the differentiation of spermatozoa in mammals. Cell Motil Cytoskeleton 1998; 41:341-52.
74. Wilson PJ, Forer A. Effects of nanomolar taxol on crane-fly spermatocyte spindles indicate that acetylation of kinetochore microtubules can be used as a marker of poleward tubulin flux. Cell Motil Cytoskeleton 1997; 37:20-32.
75. Cassimeris L, Spittle C. Regulation of microtubule-associated proteins. Int Rev Cytol 2001; 210:163-226.
76. Boucher D, Larcher JC, Gros F, Denoulet P. Polyglutamylation of tubulin as a progressive regulator of in vitro interactions between the microtubule-associated protein Tau and tubulin. Biochemistry 1994; 33:12471-7.
77. Larcher JC, Boucher D, Lazereg S, Gros F, Denoulet P. Interaction of kinesin motor domains with alpha- and beta-tubulin subunits at a tau-independent binding site: Regulation by polyglutamylation. J Biol Chem 1996; 271:22117-24.
78. Bonnet C, Boucher D, Lazereg S, Pedrotti B, Islam K, Denoulet P, Larcher JC. Differential binding regulation of microtubule-associated proteins MAP1A, MAP1B, and MAP2 by tubulin polyglutamylation. J Biol Chem 2001; 276:12839-48.
79. Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proc Natl Acad Sci USA 2003; 100:4389-94.
80. Badin-Larcon AC, Boscheron C, Soleilhac JM, Piel M, Mann C, Denarier E, Fourest-Lieuvin A, Lafanechere L, Bornens M, Job D. Suppression of nuclear oscillations in *Saccharomyces cerevisiae* expressing Glu tubulin. Proc Natl Acad Sci USA 2004; 101:5577-82.
81. Erck C, Peris L, Andrieux A, Meissirel C, Gruber AD, Vernet M, Schweitzer A, Saoudi Y, Pointu H, Bosc C, Salin PA, Job D, Wehland J. A vital role of tubulin-tyrosine-ligase for neuronal organization. Proc Natl Acad Sci USA 2005; 102:7853-8.
82. Peris L, Thery M, Faure J, Saoudi Y, Lafanechere L, Chilton JK, Gordon-Weeks P, Galjart N, Bornens M, Wordeman L, Wehland J, Andrieux A, Job D. Tubulin tyrosination is a major factor affecting the recruitment of CAP-Gly proteins at microtubule plus ends. J Cell Biol 2006; 174:839-49.
83. Honnappa S, Okhrimenko O, Jaussi R, Jawhari H, Jelesarov I, Winkler FK, Steinmetz MO. Key interaction modes of dynamic +TIP networks. Mol Cell 2006; 23:663-71.
84. Scholey JM. Intraflagellar transport. Annu Rev Cell Dev Biol 2003; 19:423-43.
85. Jacobson C, Schnapp B, Banker GA. A change in the selective translocation of the Kinesin-1 motor domain marks the initial specification of the axon. Neuron 2006; 49:797-804.
86. Reed NA, Cai D, Blasius TL, Jih GT, Meyhofer E, Gaertig J, Verhey KJ. Microtubule acetylation promotes kinesin-1 binding and transport. Curr Biol 2006; 16:2166-72.
87. Schmoranzer J, Kreitzer G, Simon SM. Migrating fibroblasts perform polarized, microtubule-dependent exocytosis towards the leading edge. J Cell Sci 2003; 116:4513-9.
88. Sharp DJ, Rogers GC, Scholey JM. Microtubule motors in mitosis. Nature 2000; 407:41-7.
89. Miller RH, Lasek RJ, Katz MJ. Preferred microtubules for vesicle transport in lobster axons. Science 1987; 235:220-2.
90. Hou Y, Qin H, Follit JA, Pazour GJ, Rosenbaum JL, Witman GB. Functional analysis of an individual IFT protein: IFT46 is required for transport of outer dynein arms into flagella J Cell Biol 2007; 176:653-65.
91. Kreitzer G, Liao G, Gundersen GG. Detyrosination of tubulin regulates the interaction of intermediate filaments with microtubules in vivo via a kinesin-dependent mechanism. Mol Biol Cell 1999; 10:1105-18.
92. Liao G, Gundersen GG. Kinesin is a candidate for cross-bridging microtubules and intermediate filaments: Selective binding of kinesin to detyrosinated tubulin and vimentin. J Biol Chem 1998; 273:9797-803.
93. Dompierre JP, Godin JD, Charrin BC, Cordelieres FP, King SJ, Humbert S, Saudou F. Histone deacetylase 6 inhibition compensates for the transport deficit in Huntington's disease by increasing tubulin acetylation. J Neurosci 2007; 27:3571-83.
94. Campbell PK, Waymire KG, Heier RL, Sharer C, Day DE, Reimann H, Jaje JM, Friedrich GA, Burmeister M, Bartness TJ, Russell LD, Young LJ, Zimmer M, Jenne DE, MacGregor GR. Mutation of a novel gene results in abnormal development of spermatid flagella, loss of intermale aggression and reduced body fat in mice. Genetics 2002; 162:307-20.
95. Ikegami K, Heier RL, Taruishi M, Takagi H, Mukai M, Shimma S, Taira S, Hatanaka K, Morone N, Yao I, Campbell PK, Yuasa S, Janke C, Macgregor GR, Setou M. Loss of alpha-tubulin polyglutamylation in *ROSA22* mice is associated with abnormal targeting of KIF1A and modulated synaptic function. Proc Natl Acad Sci USA 2007; 104:3213-8.
96. Gurland G, Gundersen GG. Stable, detyrosinated microtubules function to localize vimentin intermediate filaments in fibroblasts. J Cell Biol 1995; 131:1275-90.
97. Lin SX, Gundersen GG, Maxfield FR. Export from pericentriolar endocytic recycling compartment to cell surface depends on stable, detyrosinated (glu) microtubules and kinesin. Mol Biol Cell 2002; 13:96-109.
98. Klotz A, Rutberg M, Denoulet P, Wallin M. Polyglutamylation of atlantic cod tubulin: Immunochemical localization and possible role in pigment granule transport. Cell Motil Cytoskeleton 1999; 44:263-73.
99. Kozminski KG, Diener DR, Rosenbaum JL. High level expression of nonacetylatable alpha-tubulin in Chlamydomonas reinhardtii. Cell Motil Cytoskeleton 1993; 25:158-70.
100. Gaertig J, Cruz MA, Bowen J, Gu L, Pennock DG, Gorovsky MA. Acetylation of lysine 40 in alpha-tubulin is not essential in Tetrahymena thermophila. J Cell Biol 1995; 129:1301-10.
101. Fukushige T, Siddiqui ZK, Chou M, Culotti JG, Gogonea CB, Siddiqui SS, Hamelin M. MEC-12, an alpha-tubulin required for touch sensitivity in *C. elegans*. J Cell Sci 1999; 112:395-403.
102. Corcoran LJ, Mitchison TJ, Liu Q. A novel action of histone deacetylase inhibitors in a protein aggresome disease model. Curr Biol 2004; 14:488-92.
103. Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress. Cell 2003; 115:727-38.
104. Carta S, Tassi S, Semino C, Fossati G, Mascagni P, Dinarello CA, Rubartelli A. Histone deacetylase inhibitors prevent exocytosis of interleukin-1beta-containing secretory lysosomes: Role of microtubules. Blood 2006; 108:1618-26.
105. Serrador JM, Cabrero JR, Sancho D, Mittelbrunn M, Urzainqui A, Sanchez-Madrid F. HDAC6 deacetylase activity links the tubulin cytoskeleton with immune synapse organization. Immunity 2004; 20:417-28.
106. Tran AD, Marmo TP, Salam AA, Che S, Finkelstein E, Kabarriti R, Xenias HS, Mazitschek R, Hubbert C, Kawaguchi Y, Sheetz MP, Yao TP, Bulinski JC. HDAC6 deacetylation of tubulin modulates dynamics of cellular adhesions. J Cell Sci 2007; 120:1469-79.
107. Thazhath R, Jerka-Dziadosz M, Duan J, Wloga D, Gorovsky MA, Frankel J, Gaertig J. Cell context-specific effects of the beta-tubulin glycylation domain on assembly and size of microtubular organelles. Mol Biol Cell 2004; 15:4136-47.
108. Thazhath R, Liu C, Gaertig J. Polyglycylation domain of beta-tubulin maintains axonemal architecture and affects cytokinesis in Tetrahymena. Nat Cell Biol 2002; 4:256-9.
109. Xia L, Hai B, Gao Y, Burnette D, Thazhath R, Duan J, Bre MH, Levilliers N, Gorovsky MA, Gaertig J. Polyglycylation of tubulin is essential and affects cell motility and division in Tetrahymena thermophila. J Cell Biol 2000; 149:1097-106.
110. Redeker V, Levilliers N, Vinolo E, Rossier J, Jaillard D, Burnette D, Gaertig J, Bre MH. Mutations of tubulin glycylation sites reveal cross-talk between the C termini of alpha- and beta-tubulin and affect the ciliary matrix in Tetrahymena. J Biol Chem 2005; 280:596-606.
111. Gagnon C, White D, Cosson J, Huitorel P, Edde B, Desbruyeres E, Paturle-Lafanechere L, Multigner L, Job D, Cibert C. The polyglutamylated lateral chain of alpha-tubulin plays a key role in flagellar motility. J Cell Sci 1996; 109:1545-53.
112. Million K, Larcher J, Laoukili J, Bourguignon D, Marano F, Tournier F. Polyglutamylation and polyglycylation of alpha- and beta-tubulins during in vitro ciliated cell differentiation of human respiratory epithelial cells. J Cell Sci 1999; 112:4357-66.
113. Bobinnec Y, Khodjakov A, Mir LM, Rieder CL, Edde B, Bornens M. Centriole disassembly in vivo and its effect on centrosome structure and function in vertebrate cells. J Cell Biol 1998; 143:1575-89.
114. Palazzo A, Ackerman B, Gundersen GG. Cell biology: Tubulin acetylation and cell motility. Nature 2003; 421:230.
115. Roll-Mecak A, Vale RD. The *Drosophila* homologue of the hereditary spastic paraplegia protein, spastin, severs and disassembles microtubules. Curr Biol 2005; 15:650-5.
116. Hazan J, Fonknechten N, Mavel D, Paternotte C, Samson D, Artiguenave F, Davoine CS, Cruaud C, Durr A, Wincker P, Brottier P, Cattolico L, Barbe V, Burgunder JM, Prud'homme JF, Brice A, Fontaine B, Heilig B, Weissenbach J. Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. Nat Genet 1999; 23:296-303.
117. McNally FJ, Vale RD. Identification of katanin, an ATPase that severs and disassembles stable microtubules. Cell 1993; 75:419-29.
118. White SR, Evans KJ, Lary J, Cole JL, Lauring B. Recognition of C-terminal amino acids in tubulin by pore loops in Spastin is important for microtubule severing. J Cell Biol 2007; 176:995-1005.
119. Lu C, Srayko M, Mains PE. The *Caenorhabditis elegans* microtubule-severing complex MEI-1/MEI-2 katanin interacts differently with two superficially redundant beta-tubulin isotypes. Mol Biol Cell 2004; 15:142-50.
120. Sharma N, Bryant J, Wloga D, Donaldson R, Davis RC, Jerka-Dziadosz M, Gaertig J. Katanin regulates dynamics of microtubules and biogenesis of motile cilia. J Cell Biol 2007; In press.
121. Tarrade A, Fassier C, Courageot S, Charvin D, Vitte J, Peris L, Thorel A, Mouisel E, Fonknechten N, Roblot N, Seilhean D, Dierich A, Hauw JJ, Melki J. A mutation of spastin is responsible for swellings and impairment of transport in a region of axon characterized by changes in microtubule composition. Hum Mol Genet 2006; 15:3544-58.
122. Orso G, Martinuzzi A, Rossetto MG, Sartori E, Feany M, Daga A. Disease-related phenotypes in a *Drosophila* model of hereditary spastic paraplegia are ameliorated by treatment with vinblastine. J Clin Invest 2005; 115:3026-34.
123. Trotta N, Orso G, Rossetto MG, Daga A, Broadie K. The hereditary spastic paraplegia gene, spastin, regulates microtubule stability to modulate synaptic structure and function. Curr Biol 2004; 14:1135-47.
124. Goshima G, Wollman R, Goodwin SS, Zhang N, Scholey JM, Vale RD, Stuurman N. Genes required for mitotic spindle assembly in *Drosophila* S2 cells. Science 2007; 316:417-21.
125. Mack GJ, Compton DA. Analysis of mitotic microtubule-associated proteins using mass spectrometry identifies astrin, a spindle-associated protein. Proc Natl Acad Sci USA 2001; 98:14434-9.

126. Martin-McCaffrey L, Willard FS, Pajak A, Dagnino L, Siderovski DP, D'Souza SJA. RGS14 is a microtubule-associated protein. Cell Cycle 2005; 4:953-60.
127. Sauer G, Korner R, Hanisch A, Ries A, Nigg EA, Sillje HHW. Proteome analysis of the human mitotic spindle. Mol Cell Proteomics 2005; 4:35-43.
128. Sawin KE, Mitchison T. Mutations in the kinesin-like protein Eg5 disrupting localization to the mitotic spindle. Proc Natl Acad Sci USA 1995; 92:4289-93.
129. Lafanechere L, Courtay-Cahen C, Kawakami T, Jacrot M, Rudiger M, Wehland J, Job D, Margolis RL. Suppression of tubulin tyrosine ligase during tumor growth. J Cell Sci 1998; 111:171-81.
130. Mialhe A, Lafanechere L, Treilleux I, Peloux N, Dumontet C, Bremond A, Panh MH, Payan R, Wehland J, Margolis RL, Job D. Tubulin detyrosination is a frequent occurrence in breast cancers of poor prognosis. Cancer Res 2001; 61:5024-7.
131. Ozols J, Caron JM. Posttranslational modification of tubulin by palmitoylation: II. Identification of sites of palmitoylation. Mol Biol Cell 1997; 8:637-45.
132. Zambito AM, Wolff J. Palmitoylation of tubulin. Biochem Biophys Res Commun 1997; 239:650-4.
133. Caron JM, Vega LR, Fleming J, Bishop R, Solomon F. Single site alpha-tubulin mutation affects astral microtubules and nuclear positioning during anaphase in *Saccharomyces cerevisiae*: Possible role for palmitoylation of alpha-tubulin. Mol Biol Cell 2001; 12:2672-87.
134. Fischle W, Tseng BS, Dormann HL, Ueberheide BM, Garcia BA, Shabanowitz J, Hunt DF, Funabiki H, Allis CD. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature 2005; 438:1116-22.