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Emerging roles for tubulin PTMs in neuronal function and neurodegenerative disease

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Neurons are equipped with microtubules of different stability with stable and dynamic domains often coexisting on the same microtubule. While dynamic microtubules undergo random transitions between disassembly and assembly, stable ones persist long enough to serve as platforms for tubulin-modifying enzymes (known as writers) that attach molecular components to the α- or β-tubulin subunits. The combination of these posttranslational modifications (PTMs) results in a "tubulin code," dictating the behavior of selected proteins (known as readers), some of which were shown to be crucial for neuronal function. Recent research has further highlighted that disturbances in tubulin PTMs can lead to neurodegeneration, sparking an emerging field of investigation with numerous questions such as whether and how tubulin PTMs can affect neurotransmission and synaptic plasticity and whether restoring balanced tubulin PTM levels could effectively prevent or mitigate neurodegenerative disease.

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Introduction

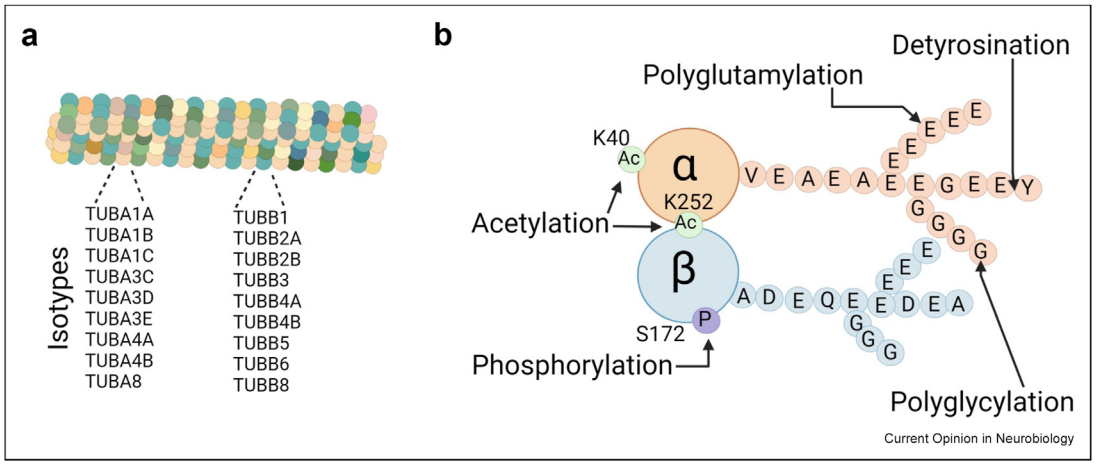
Microtubules (MTs) are abundant cytoskeletal structures serving critical roles in neuronal function, including axon pathfinding and regeneration, structural support for the formation of neuronal compartments, organelle positioning and long-distance transport, and synaptic plasticity [1–5]. While the basic building blocks of MTs are the α/β tubulin heterodimers, consisting of the combination of 9 α- and 9 β-tubulin isotypes in mammals, their functional versatility and diversity owes to an intricate array of posttranslational modifications (PTMs) that decorate either the α or β tubulin subunits residing preferentially on long-lived or highly stable MTs (Figure 1a). The combinatorial nature of these PTMs, most notably detyrosination, acetylation, and polyglutamylation (Figure 1b), generate a "tubulin code", with demonstrated modulatory roles on a few critical neuronal regulators, including MT-associated proteins (MAPs), MT-dependent motors, and MT-severing enzymes [6,7]. Therefore, the grand orchestration of relative levels of dynamic (unmodified) and stable (modified) MTs requires tight regulation by a complex tubulin-modifying machinery, consisting of enzymes responsible for the addition (writers) or removal (erasers) of tubulin PTMs when needed. Understanding the enzymatic landscape governing abundance and localization of tubulin PTMs is crucial for unraveling the intricacies of MT-mediated cellular processes and holds significant implications for various physiological and pathological conditions. For instance, several independent research groups have now confirmed the role of unmodified dynamic MTs in synaptic maintenance and their regulation by neuronal activity [4,5,8–13]. However, while the influence of tubulin PTMs associated with more stable MTs has been partially investigated in neuronal development and integrity of sensory neurons [14], whether and how tubulin PTMs might be regulated by neuronal activity or establish a code for orchestrating complex functions at synapses remains largely unexplored.

MTs are implicated in both neurodevelopment and neurodegenerative disease. In this minireview, we discuss emerging roles of selected tubulin PTMs on both postsynaptic and presynaptic functions within the adult central nervous system (CNS) and in neurodegenerative disease contexts, adding to our understanding of the pleiotropic potential of tubulin damage in neurological disease.

The tubulin deTyr/Tyr cycle

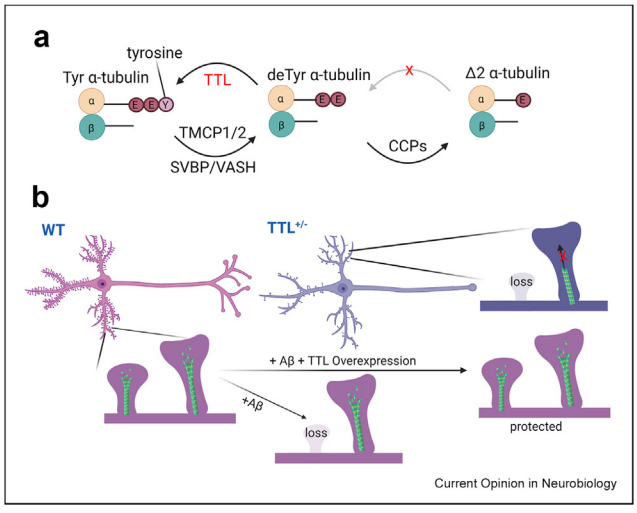
Among all tubulin PTMs, the tubulin detyrosination and re-tyrosination (deTyr/Tyr) cycle is perhaps the best understood, being primarily regulated by multiple tubulin carboxypeptidases (VASH1/SVBP and

Figure 1



(a) MTs are built from α/β tubulin heterodimers, combining 9 α-tubulin and 9 β-tubulin isotypes in mammals, each with distinctive C-terminal tails. **(b)** Tubulin heterodimer composed of α and β tubulins. The combinatorial nature of posttranslational modifications of tubulin such as detyrosination, acetylation, polyglutamylation, polyglycylation and phosphorylation create a "tubulin code" that regulates several MT-dependent neuronal functions.

Figure 2



(a) The deTyr/Tyr cycle is controlled by VASH1/SVBP or TMCP1/2, which detyrosinate α-tubulin on MTs by removing the terminal tyrosine (Y), and TTL, which re-tyrosinates α-tubulin from the soluble pool. Enzymes of the family of tubulin carboxypeptidases (CCPs) can further remove the last residue of glutamate leading to Δ2 tubulin formation. Because this reaction is not reversible, Δ2 permanently removes tubulin from the cycle. **(b)** Reduced TTL expression leads to a decrease in dendritic spine number and axonal branching *in vitro* and *in vivo*. Conversely, TTL overexpression protects dendritic spines from loss induced by oligomeric amyloid-β peptide on cultured hippocampal neurons.

MATCAP) [15,16] that catalyze the reversible cleavage of the last residue of tyrosine on the α-tubulin subunit leading to detyrosinated MTs (Figure 2a). More recently, Nicot et al. discovered a MATCAP homolog that acts on the βI-tubulin C-terminus to produce βIΔ3 mostly on centrioles and in cilia [17]. Due to their tubulin metallocarboxypeptidase activity, both enzymes are now grouped as one protein family and renamed TMCP1 (MATCAP) and TMCP2.

Notably, the deTyr/Tyr cycle is reversible: detyrosinated tubulin can be rapidly re-tyrosinated by the tubulin tyrosine ligase (TTL) thus rejuvenating the tubulin pool available for polymerization. Adding to the complexity, the cycle is regulated by intracellular calcium levels [18] and longer-lived detyrosinated MTs can be further cleaved by a family of carboxypeptidases (CCP1, 4 and 6), leading to the formation of Δ2 and Δ3 tubulins, which can no longer be re-tyrosinated [19]. It follows that while detyrosinated and Δ2 tubulins accumulate on hyperstable MTs, tyrosinated α-tubulins are abundant on highly dynamic MTs. Detyrosination of tubulin may itself promote MT stability by protecting MTs from the depolymerizing activity of kinesin-13 motors [21]. In neurons, detyrosinated MTs regulate the bidirectional trafficking of cargos, axon outgrowth, branching [22–27], and synaptic activity [28,29]. Detyrosinated MTs preferentially bind kinesin-1 and kinesin-2 [30,31] and other MAPs, allowing tyrosination-dependent loading of selected cargoes and MT modulators [32,33]. Moreover, detyrosinated MTs may serve as positive regulators of MT-severing enzymes [34]. It is then not surprising that complete disruption of the deTyr/Tyr tubulin cycle leads to severe neurodevelopmental phenotypes in the CNS of mice at birth [15,23,24,35].

Beyond their role in axon outgrowth and branching [36], detyrosinated MTs also govern the trafficking of cargos to and from synapses. Indeed, kinesin-1 participates in mitochondrial trafficking [37], in the targeting of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to dendrites [38], and in AMPA receptor-mediated synaptic transmission [39]. KIF5/kinesin-1 may additionally modulate inhibitory transmission by facilitating the transport of gamma-aminobutyric acid (GABA) receptors via huntingtin-associated protein 1 [40,41]. Furthermore, efficient kinesin-2 motility requires detyrosination [26], and homodimeric KIF17/kinesin-2 has been implicated in the transport of GluN2B, whereas disruption of KIF17/kinesin-2 impairs long-term potentiation (LTP), long-term depression, and cAMP response element-binding protein responses in mice [42]. Thus, it is not surprising that fluctuations of detyrosinated tubulin in synaptosomal fractions from the hippocampus and corresponding MT instability/stability phases are related to associative learning and memory consolidation [43]. Aged mice fail to regulate learning-dependent MT instability/stability phases and pharmacological disruption of either of the two phases leads to deficits in memory formation [43]. These data clearly indicate that failure in regulating the deTyr/Tyr cycle occurs with aging and may play a role in loss of synaptic plasticity in neurodegenerative disease by affecting synaptic MT dynamics.

Interestingly, soluble oligomeric Aβ, the peptide that accumulates in the brain in Alzheimer's disease (AD), generates subsets of detyrosinated MTs, and this accumulation of detyrosinated MTs alone is sufficient to hyperphosphorylate tau and induce tau-dependent synaptotoxicity in cultured hippocampal neurons [44,45]. These observations paved the way for the analysis of tubulin PTMs in synaptic damage and for the identification of tau-specific kinases and/or phosphatases that are modulated by tubulin PTMs. They also suggested that unbuffered fluctuations in detyrosinated tubulin might interfere with learning and memory formation and may be targeted for therapeutic intervention. Further supporting this model, we recently reported that loss of tubulin re-tyrosination catalyzed by the TTL, the tubulin "rejuvenating" enzyme (Figure 2a), causes synaptic plasticity and memory deficits in TTL hemizygous mice and is a feature of familial and sporadic AD [29]. These results demonstrated that tubulin re-tyrosination regulates synaptic plasticity *in vivo*, is protective against dendritic spine loss (Figure 2b), and is perturbed in AD, providing strong support for a pathogenic role of defective tubulin modification in neurodegenerative disease. The contribution of Δ*2* accumulation to these phenotypes remains unclear, although evidence from a toxic form of peripheral neuropathy suggests a pathogenic role for excessive Δ*2* in promoting axon degeneration [20]. Likewise, Hosseini et al. reported that the conditional removal of TTL in hippocampal CA1 excitatory neurons decreased the response to theta-burst stimulation-induced LTP and the postsynaptic spine density on dendrites [28]. Notably, this alteration did not impact basal synaptic transmission, underscoring a role for this tubulin PTM for activity-dependent plasticity in excitatory synapses. In addition, the deletion of TTL in the forebrain, encompassing the neocortex and hippocampus, halts axon growth, affecting structures like the corpus callosum and anterior commissures [28]. Together, these discoveries provide valuable insights into molecular mechanisms involved in memory formation and maintenance, while emphasizing compromised synaptic plasticity and reduced density of postsynaptic terminal spines in association with disruptions in the deTyr/Tyr cycle.

Excessive tubulin detyrosination was reported to promote tau hyperphosphorylation in murine hippocampal neurons [45]. Conversely, disrupting polyglutamylation was shown to prevent the accumulation of hyperphosphorylated tau in mice [46]. Interestingly, a recent *in vitro* study by Ebberink et al. has demonstrated that an extended polyglutamate chain on semi-synthetic MTs enhances detyrosination activity of SVBP, suggesting that tubulin polyglutamylation may contribute to tau hyperphosphorylation through its effect on tubulin detyrosination [47]. Notably, old mice accumulate more tubulin polyglutamylation in the hippocampus than their younger counterparts [48]. It is tempting to speculate that in addition to TTL loss, disruption of the deTyr/Tyr cycle in AD may also be influenced by extended tubulin polyglutamate chains and potential impairment of glutamate homeostasis. Future research is needed to deepen our understanding of this crosstalk and determine whether these potentially synergistic effects can be leveraged for the development of alternative therapeutic interventions.

Tubulin acetylation

In mammals, tubulin can be acetylated at several residues. Tubulin acetylation and deacetylation can occur on lysine 40 of α-tubulin and is facilitated predominantly by two soluble enzymes: the α-tubulin N-acetyltransferase 1 (αTAT1 or ATAT1) and the histone deacetylase 6 (HDAC6) [49]. The acetyltransferase ATAT1 adds acetyl groups on α-tubulin subunits residing on long-lived MTs, while HDAC6 removes them. What sets this modification apart from the others is its occurrence on the luminal surface of MTs, contributing to the formation of sturdy, enduring MT structures that decrease MT turnover and increase resistance to depolymerization [50,51]. Hence, although tubulin acetylation takes place on pre-existing stabilized MTs as a luminal modification, it itself can enhance the stability of the MT lattice by providing

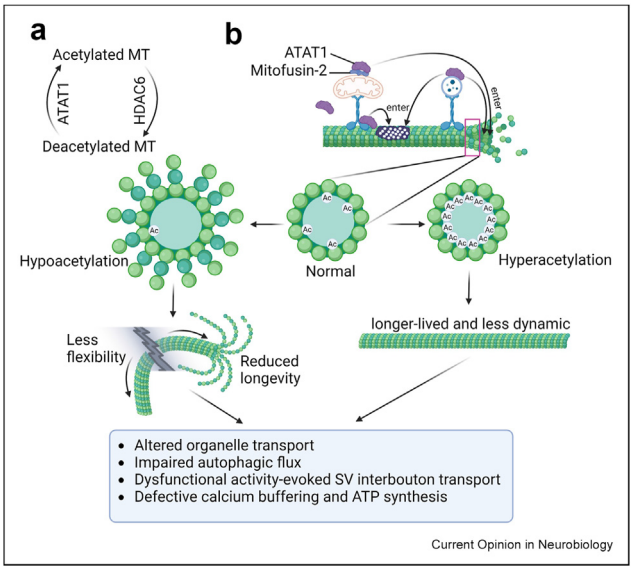
resistance to mechanical breakage [51]. These characteristics likely contribute to the accumulation of acetylated MTs within stable MT networks in neuronal processes, resulting in heightened stability. Resistance to mechanical breakage might also provide an explanation as to why an intraluminal tubulin modification may impact on microtubule-dependent organelle transport. In cells, acetylated MTs were shown to serve as the favored tracks for kinesin-1 [52,53], the primary motor protein involved in plus-end oriented transport, and are utilized as tracks for kinesin-1-mediated transport of mitochondria, thereby facilitating contacts with the endoplasmic reticulum, as well as mitochondrial fusion and fission events [54–56].

Alterations in K40 acetylation on α-tubulin have been documented in conditions such as oxidative stress, neuropathic disorders, and neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease (PD). This is not unexpected, given that approximately 30 % of brain tubulin is acetylated [50], and quantitative super-resolution imaging has revealed that in dendrites, nearly 80 % of all neuronal MTs exhibit varying degrees of acetylation [57]. Despite this compelling evidence, the physiological role of tubulin acetylation in neurons remains largely undefined, except in sensory neurons, where it appears to play a critical role in the mechanosensory machinery by regulating cellular stiffness [58,59].

An increase in α-tubulin acetylation has been shown to help maintain the structure and function of dendritic spines following ischemic stroke [60], suggesting that the impact of MT acetylation on synapses may be primarily related to structural maintenance. Nevertheless, the role of tubulin acetylation in synaptic function is far from understood. The role of acetylated tubulin in neuronal morphogenesis is linked to molecular effectors like CAMSAP3 (calmodulin-regulated spectrin-associated protein 3), which specifically interacts with non-acetylated MTs to regulate MT minus-end dynamics [61], as well as to the MT-severing by katanin, which tends to occur at acetylated MT sites in dendrites [62]. It remains to be determined whether these properties impact on tubulin acetylation-dependent modulation of synapses.

MT acetylation occurs within the structural lumen of MTs, and accessing this lumen is a rate-limiting step for the enzymes involved, such as HDAC6 and ATAT1 (Figure 3a). Interestingly, in non-neuronal cells, HDAC6 can enter the MT lumen through kinesin-1-induced damage sites, resulting in an acetylation gradient that is higher near the nucleus than at the cell periphery [63]. Although this analysis has not been conducted in neurons, a tubulin tyrosination gradient has been reported in long axonal processes, suggesting coexistence of an opposing acetylation gradient

Figure 3



(a) Acetylation and deacetylation of K40 on α-tubulin are regulated by ATAT1 and HDAC6 respectively. **(b)** In neurons, ATAT1 associates with axonal vesicles and mitochondria to facilitate entry into the MT lumen at lattice cracks or MT ends. MT hypoacetylation decreases MT stability, while hyperacetylation is predicted to enhance it. These modifications may influence organelle transport, autophagic flux, activity-evoked SV interbouton transport, and organelle-mediated local calcium buffering or ATP synthesis.

associated with more stable MTs [64,65]. It is tempting to speculate that a tubulin acetylation gradient could be relevant to the differences in synaptic function between proximal and distal processes [66], especially when the synapses are located far from the nucleus. For instance, this gradient could influence the efficiency of synaptic cargo targeting and activity, making distal synapses weaker than proximal ones.

In neurons, ATAT1 has been observed on the cytosolic side of vesicles that move along axons, suggesting that axonal membrane cargos may have on-board enzymatic machinery that acts locally to modulate their long-distance transport by increasing the flexibility and resistance of their own MT tracks through tubulin acetylation [67]. By analogy with axonal vesicles, mitochondria can also promote α-tubulin acetylation by recruiting ATAT1 to their MT contact sites and loss of this activity may underlie predominantly axonal form of Charcot–Marie–Tooth disease, which is characterized by axon degeneration [68]. It remains to be determined whether this activity is preserved also in CNS neurons. Additionally, while MT hypoacetylation is predicted to affect axonal integrity and synaptic function indirectly by reducing MT resistance to mechanical compression and axonal transport [50,51,68], the consequences of MT hyperacetylation on axonal integrity and presynaptic release have not been investigated (Figure 3b). This

is a significant question for several reasons. First, tubulin hyperacetylation might lead to premature tubulin longevity and accumulation of tubulin PTMs, which would disrupt the balance between kinesin (anterograde transport) and dynein (retrograde transport) binding to MTs, with negative consequences for organelle trafficking, contacts, and turnover. Second, tubulin hyperacetylation in the axon may directly inhibit the dynamicity of MTs at presynaptic terminals required for activity-evoked interbouton synaptic vesicle (SV) transport and disrupts a tubulin acetylation gradient leading to impaired neurotransmitter release and synaptic strength [69]. Third, mitochondrial and presynaptic activities have a strong functional relationship and altered mitochondrial dynamics caused by tubulin hyperacetylation that could indirectly affect SV cycling and presynaptic release. Indeed, the number of docked vesicles positively correlates with presynaptic mitochondria [70—72] and mitochondrially derived ATP is required to sustain neurotransmission during elevated levels of stimulation [73,74]. In addition, mitochondria regulate presynaptic release by sequestering cytosolic Ca^{2+} or altering ATP concentrations [75—78]. Additional effort is required to explore these possibilities.

Regulation of tubulin acetylation in AD and PD remains highly controversial. In AD, examining tubulin acetylation levels in post-mortem human brain samples have produced mixed results. Some research indicates a decrease in acetylated tubulin in AD, which correlates with higher levels of HDAC6 [79—81]. Conversely, other studies suggest that while the overall MT mass decreases in AD, the proportion of acetylated tubulin actually increases [82,83]. Interestingly, HDAC6 contributes to AD-related features, potentially through its interaction with tau [83—85]. However, oligomeric Aβ (oAβ) inhibits HDAC6 [86], while causing accumulation of acetylated tubulin in hippocampal neurons [45]. We recently reported that tubulin acetylation accumulates in post-mortem brain tissues from AD patients and in human neurons carrying the Alzheimer's familial APP-V717I mutation [87]. Moreover, we provided evidence that tubulin re-tyrosination, which is impaired in AD, can regulate acetylated tubulin in primary neurons independently of the levels of the enzymes involved in tubulin acetylation. This suggests that the reduced MT dynamics associated with defective tubulin re-tyrosination may alone contribute to the observed accumulation of tubulin acetylation in Alzheimer's disease. Whether this crosstalk may play a pathophysiological role in AD remains to be validated.

In PD, the PD inducing neurotoxin 1-methyl-4-phenylpyridinium (MPP+), a metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), causes tubulin hyperacetylation, and MT dysfunction always precedes defects in mitochondrial dynamics promoted by this drug [88]. An increase in tubulin acetylation is also observed in dopaminergic terminals of the *corpus striatum* and in the cell bodies of dopamine neurons located in the substantia nigra pars compacta (SNc DA neurons) of mice injected with MPTP [89]. Strikingly, strong accumulation of acetylated tubulin has been recently reported in neuronal cell bodies in the subcortical areas affected by immature Lewy body (LB) pathology, suggesting that α-tubulin hyperacetylation may occur in an early temporal window and contribute to the final aggregated form of α-synuclein and LB biogenesis [90]. Finally, tubulin hyperacetylation was also shown in the context of familial PD-associated proteins, including LRRK2 (PARK8) and parkin (PARK2) [91,92]. LRRK2 directly interacts with β-tubulin at a site close to the acetylated K40 residue on α-tubulin, suggesting that LRRK2 can regulate acetylated tubulin levels [93]. However, while overexpression of mutated forms of LRRK2 reduces acetylated tubulin and axonal transport [94], both embryonic fibroblasts derived from LRRK2 knock out (KO) mice and skin fibroblasts isolated from PD patients with LRRK2 mutations display tubulin hyperacetylation [93,95]. This suggests that tubulin hyperacetylation resulting from loss of LRRK2 function, rather than tubulin hypoacetylation caused by LRRK2 overexpression, may drive the disease phenotype. Mutations in DJ-1 (PARK7) in PD remain poorly characterized, although a role for DJ-1 deficiency in mitochondrial dysfunction and in MT dynamics inhibition has been shown [96,97]. Despite this compelling evidence; however, it remains unclear which mechanisms lead to MT changes in PD and whether alterations in MT dynamics and tubulin PTMs are primary or secondary events in the induction of neurodegeneration of SNc DA neurons.

Tubulin polyglutamylation

Tubulin glutamylation, both monoglutamylation and polyglutamylation, represents another reversible PTM that is notably abundant in adult neurons. This modification generates chains of glutamate of varying lengths through the action of several tubulin tyrosine ligase-like (TTLL) glutamylases and polyglutamylases (TTLL1, 4, 5, 6, 7, 9, 11, and 13) acting on glutamic acid residues located on the C-terminal tails of either α- or β-tubulin [98—100].

The CCP family of cytosolic carboxypeptidases (CCP1, 2, 3, 4, and 6) is responsible for removing the added glutamate residues, maintaining homeostatic polyglutamylated tubulin levels [101,102]. The *in vivo* impact of tubulin hyperglutamylation was initially highlighted by the discovery that the Purkinje cell degeneration (pcd) mouse model [103] exhibits a spontaneous mutation in the *Nna1* encoding gene (renamed later *Ccp1* or *AGTPBP1*) [104], resulting in

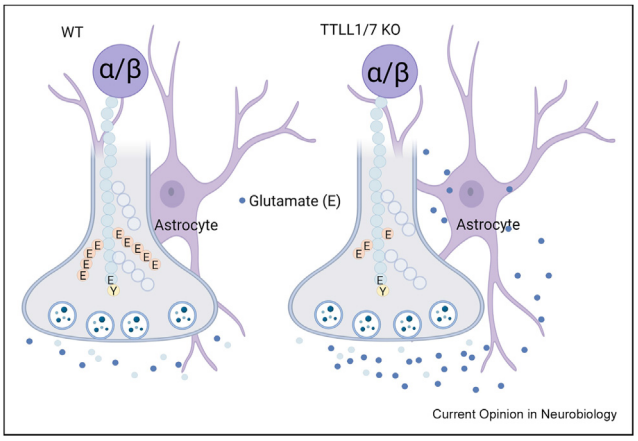
various neuronal abnormalities and severe cerebellar ataxia characterized by rapid degeneration of Purkinje cells. This degeneration is attributed to a significant accumulation of polyglutamylated tubulin in the cerebellum as shown in the mouse [48,101], and a similar pathology was found in humans with a rare form of *Ccp1* inactivation [105]. At the cellular level, although multiple microtubule-dependent mechanisms are predicted to contribute to this phenotype, disrupted organelle trafficking and impaired mitochondrial dynamics emerge as common abnormalities caused by tubulin hyperglutamylation and are likely key drivers of neurodegeneration [48,98,99,106].

A role for synaptic function in regulating a tubulin PTM was initially reported in the case of tubulin polyglutamylation. In this study, the pharmacological activation of AMPARs or the inhibition of glycine receptors (GlyR)-mediated inhibitory neuronal activity enhanced tubulin polyglutamylation, leading to increased interaction between MAP2 and MTs, as well as reduced mobility of motor protein-mediated cargo delivery into dendrites [107]. Interestingly, the neuron-specific β-tubulin Tubb3 is also sensitive to changes in neuronal activity. In addition, Tubb3 expression appears to regulate tubulin polyglutamylation levels [108,109], suggesting the existence of signaling pathways that connect neuronal activity with tubulin isotype expression and the enzymes of the glutamylation/deglutamylation cycle.

Transitioning to presynaptic sites, we predict that the deTyr/Tyr tubulin cycle works in both excitatory and inhibitory presynapses, given that the axonal trafficking of N-methyl-D-aspartate (NMDA) and $GABA_A$ receptors depend on kinesin-1 and kinesin-2 [30,31]. CCP1 is responsible to generate Δ2-tubulin when TTL re-tyrosination level is low [20]. On the other hand, CCP1 also increases polyglutamylation levels, potentially leading to changes in presynaptic functionality by impairing axonal organelle trafficking in neurons [98]. Knocking out TTLL1, a polyglutamylase specific to α-tubulin, reduces the level of polyglutamylation in *Ccp1* KO mice, potentially restoring normal MT dynamics and protecting cerebellar Purkinje cells from degeneration [99]. Notably, TTLL1 KO alone does not increase Δ2-tubulin [99], suggesting that CCP1's ability to generate Δ2-tubulin is TTL-dependent. Moreover, tubulin polyglutamylation leads to disruption in kinesin-1 transport and a decrease in the number of presynaptic sites in motor neurons [110]. Despite the implication of tubulin hyperglutamylation in impaired axonal transport, neurodegenerative disease, and circuitry integrity [110–112], it remains to be established whether tubulin polyglutamylation directly affects synaptic function.

One speculative model posits that tubulin polyglutamylation may function as a reservoir for glutamate,

Figure 4



TTLL1/7 KO reduces MT polyglutamylation and increases extracellular glutamate levels, suggesting that TTLL1/7 KO neurons may experience increased calcium influx. Elevated extracellular glutamate may influence astrocytes, which play a critical role in maintaining glutamate homeostasis by absorbing excess glutamate and converting it to glutamine for recycling. In contrast, CCP1/6 KO neurons, which have increased MT polyglutamylation, are expected to have reduced calcium influx.

the primary excitatory neurotransmitter in the brain. Within neurons, glutamate is generated through pathways such as the glutamine–glutamate cycle and the GABA shunt pathway involving astrocytes, as well as neuronal endogenous transamination processes, including mitochondrial reactions and glucose metabolism via the tricarboxylic acid (TCA) or Krebs cycles [113]. Once synthesized, glutamate is actively transported into vesicles within presynaptic terminals by vesicular glutamate transporters (VGLUTs). Upon neuronal activation, it is released into the synaptic cleft, binding to postsynaptic receptors, such as NMDA receptors, to initiate excitatory signaling and synaptic transmission. While synaptic vesicles provide temporary storage, glutamate can also be synthesized on-demand from precursor molecules including glutamine, α-ketoglutarate, GABA, and aspartate, with its availability at synapses closely linked to local mitochondria energy metabolism [113]. Recent evidence from Ping et al. suggests that tubulin C-terminal polyglutamate chains may contribute to a reservoir for glutamate. Using TTLL1 and TTLL7 knockout mice, which exhibit reduced polyglutamylation of α- and β-tubulins, respectively, along with matrix-assisted laser desorption/ionization imaging mass spectrometry to visualize glutamate distribution in the brain, they found that the loss of TTLL1 and TTLL7 increased glutamate concentration (Figure 4), particularly in the cortex region where polyglutamylated tubulin levels were decreased [114]. These findings suggest that tubulin polyglutamylated chains may serve as buffering reservoirs, with their breakdown leading to extracellular glutamate

overflow. This raises questions about whether the loss of MT polyglutamylation affects mitochondrial energy metabolism at the presynapse and influences neurotransmitter availability at the presynapse and postsynapses, indicating a potential MT role beyond structural maintenance and cargo transport.

Tubulin phosphorylation and poly-glycylation

Tubulin phosphorylation is another crucial regulator of MT dynamics and phosphorylation of β-tubulin at serine 172 (S172) by the cyclin-dependent kinase Cdk1 is the best-characterized example. Moreover, a recent study in human Induced pluripotent stem cells (IPSC)-derived astrocytes further links β-tubulin phosphorylation to impaired vesicle trafficking and mitochondria cargo delivery in a PANK2-associated neurodegeneration disease model [115]. This study also observed a two-fold increase in S172-phosphorylated β-tubulin together with a 2-to-4-fold reduction in K40-acetylated α-tubulin, raising the interesting possibility of reciprocal regulation. Since S172-phosphorylated β-tubulins are unable to incorporate into MTs [116], and given that ATAT1, preferentially acetylates polymerized MTs [117], an increase in S172-phosphorylated β-tubulin may limit the available substrate for ATAT1, leading to decreased K40 acetylation. Further supporting this, Lu et al. demonstrated that S172 phosphorylation significantly affects MT stability and neurite development in *C. elegans* neurons [118]. Specifically, the phosphomimicking S172E mutation inhibits neurite growth by blocking MT stabilization while increasing MT dynamics and disrupting MT polarity. In contrast, the non-phosphorylatable S172A mutation enhances MT stability and reduces MT dynamics, resulting in excessive neurite outgrowth without affecting MT polarity. The underlying consequences of this tubulin modification are yet to be fully elucidated although a role for S172 phosphorylation has been proposed in the regulation of mitochondria and SV transport [118].

Another notable phosphorylation event in tubulin occurs on β3-tubulin, a neuronal specific isotype in mammals, by casein kinase II (CK2α), citron kinase (Cit-K), and G-protein-coupled receptor kinase 2 [119–121]. The phosphorylation at S444 has been shown to inhibit MAP2-mediated MT assembly *in vitro* [122] and is functionally required for the cytokinesis of neuronal progenitor cells [120]. However, its specific role in mature neurons and whether additional tubulin phosphorylation sites might add to the complexity remains largely unexplored.

Tubulin glycylation (mono- or poly-glycylation) occurs when glycine chains of varying lengths are attached as side chains to the γ-carboxyl of a glutamate residue at the C-terminus of an α or β-tubulin, thus competing with glutamylation for the same glutamate sites on tubulin. Whether this is simply a curious convergence, a case of mutual regulation, or has functional significance in neurons remains to be determined. Furthermore, compared with the relatively understudied phosphorylation, tubulin glycylation remains an even more unexplored territory. Firstly, while the enzymes responsible for adding glycine to the chain have been identified (TTLL3, TTLL8, and TTLL10) [123], the enzymes responsible for removing glycine remain completely unknown. Secondly, poly-glycylation is uniquely observed on stable MTs within primary cilia and little is known about the presence and function of tubulin glycylation on noncentrosomal MT arrays in neurons. It is important to note that various types of brain cells, including hippocampal dentate gyrus granule neurons, astroglial cells, and neuronal progenitor cells [124,125], also possess primary cilia, which suggests that this tubulin PTM may play an important role in the differentiation of both neurons and glia. Furthermore, the recent discovery of a serotonergic axo-ciliary synapse [126] might highlight the significance of exploring the roles for tubulin glycylation in neuronal communication.

Concluding remarks

Emerging evidence suggests that in addition to organelle transport, MAP binding, and severing enzyme activity, tubulin PTMs may regulate multiple synaptic functions, including dendritic spine dynamics and neurotransmitter release. Nevertheless, our understanding of their pleiotropic potential is still in its infancy, leaving many questions yet to be answered. It is also becoming evident that disruption of homeostatic tubulin PTM levels drives neurodegeneration. Interestingly, proper regulation of relative tubulin PTM levels decrease with aging [43,48]. It is tempting to speculate that in postmitotic neurons with limited cellular renewal capacity, tubulin PTMs may function as a molecular clock, thereby influencing cognitive processes and the susceptibility to age-related diseases. Therefore, targeting the tubulin "writers" or "erasers" may be beneficial for restoring circuit integrity in age-related neurodegenerative conditions. The molecular factors underlying these regulatory pathways remain to be fully identified. For instance, future studies could be designed to provide mechanistic evidence for how inhibition of tubulin re-tyrosination affects synaptic function and whether it might impact tau phosphorylation in health and disease. In addition, while depletion of tubulin re-tyrosination has been linked to synaptic dendritic spine pruning *in vitro* and *in vivo* [29], its *in vivo* impact on presynaptic function, axonal reinnervation, and branching remains elusive. Exploration of this aspect could shed light on the factors influencing the retention of learning and memory in

some older individuals while others experience decline and provide promising avenues for therapeutic intervention.

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Declaration of competing interest

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Data availability

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* of special interest
** of outstanding interest

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