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Tubulin Polymerization Promoting Proteins: Functional Diversity With Implications in Neurological Disorders

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ABSTRACT

*T*ubulin *P*olymerization *P*romoting *P*roteins (TPPPs) are highly conserved across species but remain poorly understood. There are three *TPPP* genes in humans, with only one homologous *TPPP* gene in invertebrates, such as *Drosophila* and *C. elegans*. The human TPPP (TPPP1/p25/p25α) is enriched in the brain and shares sequence similarities with the invertebrate TPPPs. TPPP/p25 associates with microtubules and plays a pivotal role in microtubule dynamics, bundling, and polymerization, thereby stabilizing the microtubular network. This is essential for cytoskeletal organization and proper functioning of neurons and glial cells, including axonal growth, regeneration, migration, trafficking, synapse formation, and myelination of axons. However, studies have also uncovered that besides its cytoplasmic/microtubular localization, TPPP/p25 is present in other subcellular compartments, including the mitochondria and nucleus, underscoring the presence of additional novel functions. At the molecular level, TPPP/p25 is predicted to exist as an intrinsically disordered protein and is implicated in neurological and neurodegenerative disorders, including Parkinson's and related disorders and Multiple Sclerosis. In this article, we provide a comprehensive overview of TPPP/p25, highlighting its evolutionary conservation, cellular and subcellular localization, established and emerging functions in the nervous system, interacting partners, potential clinical relevance to human neurological disorders, and conclude with unresolved questions and future areas of study.

1 | Introduction

Tubulin polymerization promoting proteins (TPPPs) is a family of proteins known for more than two decades and was identified during the purification of the tau protein kinase II from bovine brain extracts (Takahashi et al. 1991). The first member of the TPPP protein family to be identified was TPPP1/p25α/p25, which is a 25 kDa protein enriched in the human brain and is expressed in the nervous systems of other vertebrates and invertebrates (Takahashi et al. 1991, 1993; Seki et al. 1999; Orosz and Ovadi 2008; Ovadi and Orosz 2009). TPPP1/p25α/p25, hereafter referred to as TPPP, will be the focus of this review. Human TPPP was identified and mapped to the p15.3 region of chromosome 5 (Seki et al. 1999). TPPP is highly conserved across many species, underscoring its biological significance (Skjoerringe et al. 2006; Orosz and Ovadi 2008; Mino et al. 2016), yet is very understudied, and much remains unknown. Many of the reported studies on TPPP using various in vitro cell culture

Summary

- Tubulin polymerization promoting proteins (TPPP) are known for well over two decades but remain understudied.
- Although their most characterized function is in microtubule bundling and polymerization, emerging evidence highlights novel roles of TPPP with implications for neuronal survival.
- Human TPPP is associated with multiple nervous system disorders, including α-synucleinopathies, such as Parkinson's disease, Multiple System Atrophy, and Lewy Body Dementia, as well as Multiple Sclerosis.
- This review article provides a comprehensive overview of TPPP across species, emphasizing its functional diversity and relevance in human disease pathogenesis.

systems have provided important insight into TPPP functions (Hlavanda et al. 2002; Lehotzky et al. 2004; Tokesi et al. 2010; Ejlerskov et al. 2013; Mavroeidi et al. 2022). However, the lack of any knockout animal model of TPPP for over a decade and a half since its first identification posed a significant impediment to understanding the scope of the in vivo physiological roles of TPPP. The first in vivo knockout animal of TPPP was reported in *Drosophila melanogaster* (Mino et al. 2016) followed by the in vivo knockout of mouse TPPP (Fu et al. 2019). These knockout models have started paving the way to explore the developmental, behavioral, biochemical, and mechanistic roles of TPPP in the nervous system across invertebrate and vertebrate species. Subsequent studies on TPPP since its initial discovery that have provided key insight into its functions are summarized in Figure 1.

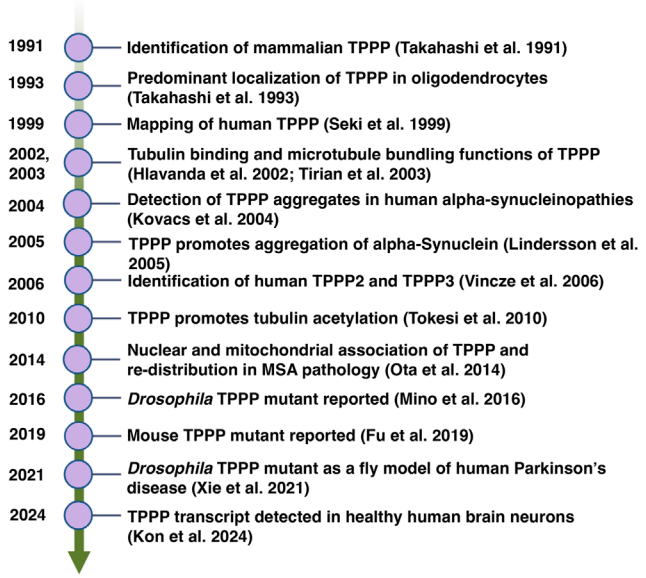

FIGURE 1 | Milestones in TPPP research. A schematic presentation of the key milestones in TPPP research since its initial discovery. Image created with BioRender.com.

TPPP plays a pivotal role in microtubule dynamics and cytoskeletal organization (Hlavanda et al. 2002; Lehotzky et al. 2004, 2010; Acevedo et al. 2007; Schofield et al. 2013). TPPP's role in microtubule bundling and polymerization is essential for the stability of the microtubular network that is required for proper axonal outgrowth, regeneration, and axonal ensheathment/myelination (Mino et al. 2016; Fu et al. 2019; Shi et al. 2019; Vargas et al. 2020). In addition to these well-known functions of TPPP in orchestrating cytoskeletal organization, studies have highlighted its presence in mitochondria and its related functions (Ota et al. 2014; Xie et al. 2021). These studies underscore the existence of additional novel roles of TPPP that may have functional implications in oxidative stress and neuronal survival. These emerging roles are relevant since TPPP is implicated in various human nervous system disorders, including neurological and neurodegenerative disorders (Kovacs et al. 2004; Lindersson et al. 2005; Hoftberger et al. 2010; Jellinger and Lantos 2010; Kon et al. 2024). In addition, TPPP is also an intrinsically disordered protein (IDP). IDPs are often associated with neurodegenerative and other disorders. This dual structure–function component of TPPP could have significant implications toward understanding the molecular mechanisms behind the human disorders.

The objective of this review article is to provide an overview of the functional diversity of TPPP, delving into its well-established functions while also shedding light on the newly identified roles within the nervous system across species. Additionally, the article seeks to explore the cellular and subcellular localizations of TPPP, the array of proteins with which it interacts with and the potential clinical significance of TPPP in human nervous system disorders.

2 | Evolutionary Conservation of TPPP

Sequence homology of TPPP proteins is seen from unicellular organisms to vertebrates (Tirian et al. 2003; Orosz 2009). The high conservation of TPPP across various species (Figure 2) underscores its crucial role in cellular processes. TPPP functions primarily as a microtubule-associated protein (MAP). Despite the long phylogenetic distance, TPPP across species possesses the same tubulin binding and polymerizing properties as human TPPP, suggestive of an evolutionarily conserved function (Štifanić et al. 2011). Interestingly, the region responsible for tubulin binding is the same in the sponge TPPP as in the human TPPP (Olah et al. 2017a). Multiple sequence alignments from TPPP proteins across diverse species highlight the highly conserved regions within the TPPP protein family, particularly emphasizing the central microtubule binding (or p25α) domain, suggesting significant functional importance of this domain (Figure 3).

TPPP is conserved in the genomes of ciliated organisms (Orosz and Ovadi 2008; Orosz 2022). Cilia and flagella are microtubule-based cellular extensions and function mainly as sensory and motile units (Linck et al. 2014). The presence of TPPP orthologs in all ciliated organisms is suggestive of their association with ciliary functions. The primary cilium, which is a sensory appendage present in most mammalian cells, including neurons and glial cells, plays critical roles in various signaling pathways.

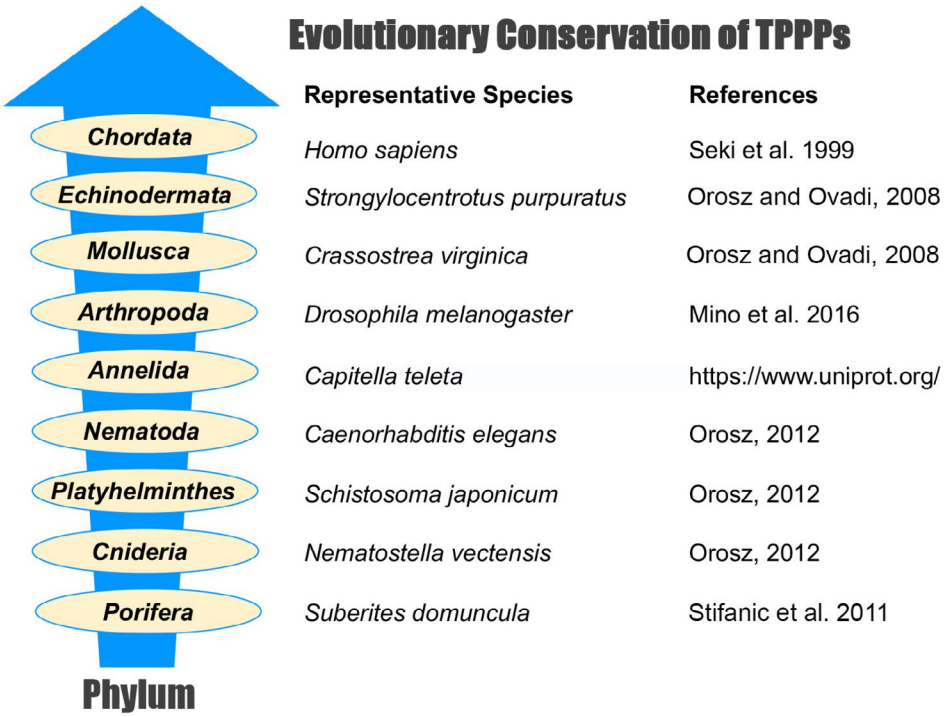

FIGURE 2 | Evolutionary conservation of TPPPs across animal phyla. TPPP is conserved across all major animal phyla from Porifera to vertebrates highlighting its essential biological functions during animal evolution.

Deficits in the structure or function of primary cilia lead to ciliopathies that encompass more than a dozen syndromes, including Joubert syndrome and Usher syndrome (Youn and Han 2018). TPPP was identified in the proteomic analysis of mouse photoreceptor sensory cilium (Liu et al. 2007) and was also downregulated along with several ciliary genes in gene expression studies carried out from ciliary dyskinesia patients (Geremek et al. 2014). These studies suggest that TPPP is present in the cilia in various organisms and might contribute to ciliopathies in humans, but their role in the cilium remains to be further explored.

There are three TPPP genes in humans, TPPP/p25 (~25 kDa), TPPP2/p18 (18 kDa), and TPPP3/p20 (20 kDa) (Vincze et al. 2006). Human TPPP and TPPP3 are known for their microtubule-associated functions (Olah et al. 2017a, 2022), whereas TPPP2 is implicated in male fertility (Liu et al. 2013; Zhu et al. 2019; Wang et al. 2021; Alagundagi et al. 2023; Orosz 2024). In invertebrates, such as *Drosophila melanogaster*, *Caenorhabditis elegans*, and *Anopheles gambiae*, there is generally one TPPP, which is ~40–48% identical with each other and with the mammalian TPPP proteins. The identity becomes considerably higher (~80%) within only the last 50 amino acids of the C-terminal sequences of the TPPP proteins (Mino et al. 2016; Olah et al. 2017a). The TPPP family of proteins across species is characterized by the presence of the p25α domain named after its first member, TPPP/p25. TPPP/TPPP-like proteins can be further grouped according to either the length of their p25α domain (such as long, short, truncated, or partial) or the presence of additional domain(s) such as EF-hand and doublecortin (Orosz 2023), and most eukaryotes contain one long TPPP (Orosz 2012). Long-type TPPPs are characterized by a Rossmann-like motif (GXGXGXXGR) in their C-termini (Figures 3 and 4) (Tirian et al. 2003; Orosz 2012). These motifs are of ancient evolutionary origin, are present in a variety of metabolic enzymes, and are capable of binding diverse ligands to perform diverse functions (Medvedev et al. 2021). The presence of the Rossmann-like sequence motif may also contribute toward the functional diversity of TPPP and potentially its relevance in certain disorders (Medvedev et al. 2019).

3 | TPPP Cellular and Subcellular Localization in the Nervous System

Understanding the cellular and subcellular localization of TPPP is crucial for deciphering its physiological and pathological roles. TPPP is enriched in the human brain and is expressed in various cell types in the nervous system across species. TPPP also exhibits diverse sub-cellular localizations, including the well-established cytoplasmic/microtubule localization as well as nuclear and mitochondrial localizations that suggest its involvement in multiple cellular processes and functions (summarized in Figure 5).

3.1 | Cellular Localization of TPPP Across Species

In humans and other vertebrates, such as mice and rats, TPPP is strongly expressed in the CNS oligodendrocytes (Takahashi et al. 1993; Song et al. 2007; Fu et al. 2019). TPPP is a constituent of myelin and colocalizes with myelin basic protein (MBP) in the healthy human brains (Song et al. 2007). In healthy human brain neurons, TPPP transcripts were detected at significant levels in both the excitatory and inhibitory neurons using RNA scope and single nuclear RNA sequencing studies.

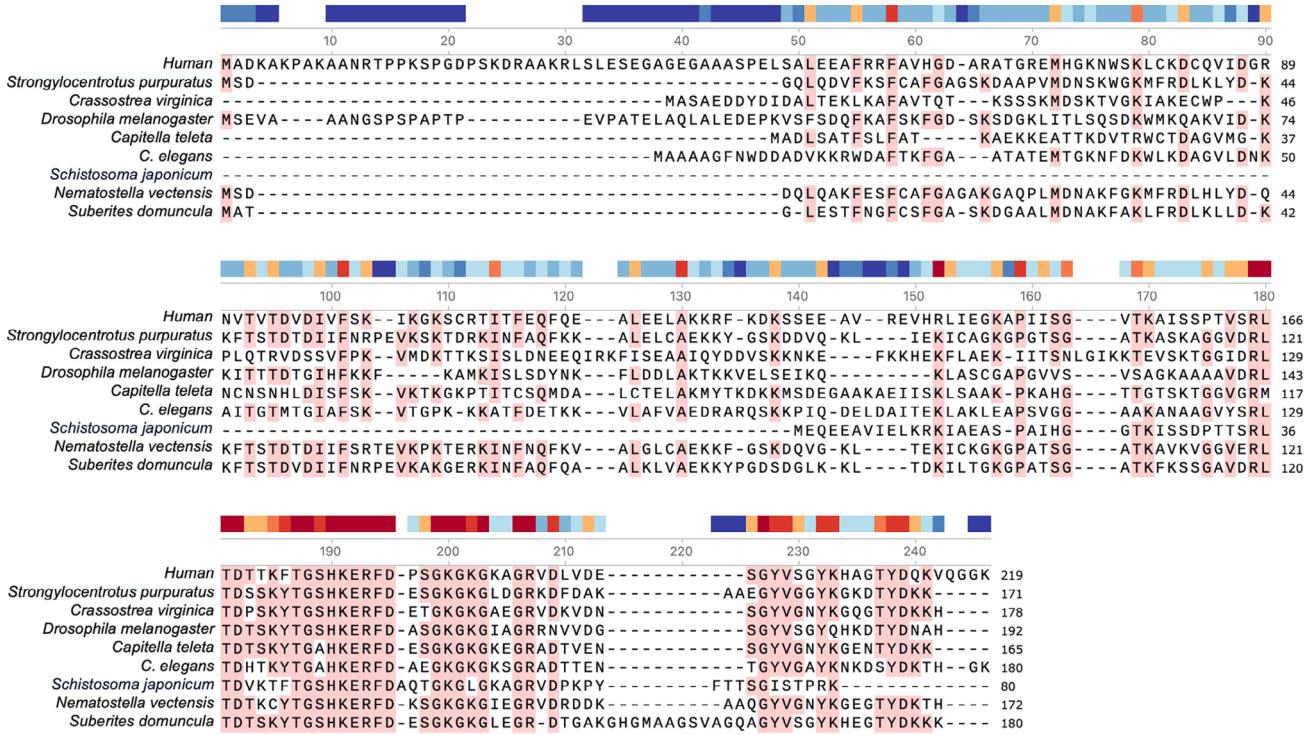

FIGURE 3 | Multiple sequence alignment of TPPP-family proteins across diverse species. Protein sequences from *human* (TPPP1), *Strongylocentrotus purpuratus, Crassostrea virginica, Drosophila melanogaster, Capitella teleta, Caenorhabditis elegans, Schistosoma japonicum, Nematostella vectensis*, and *Suberites domuncula* were aligned using SnapGene software. The alignment was performed using the integrated multiple sequence alignment tool and visualized within the SnapGene protein viewer. Highlighted residues (in yellow) indicate positions conserved among multiple species but not necessarily matching the consensus sequence. Gaps introduced to optimize the alignment are indicated by dashes (–). The colored bars above the sequences represent conservation scores: Dark red blocks indicate regions of high sequence conservation, whereas lighter blue/yellow/orange blocks represent regions of moderate to low conservation. Regions without color or with dark blue indicate no strong conservation across aligned sequences. This visualization highlights highly conserved regions within the TPPP protein family, particularly emphasizing the central microtubule binding (or p25α) domain, suggesting significant functional importance despite evolutionary divergence. Within the p25α domain, the GXGXGXXGR motif stands out as a highly conserved sequence, indicative of a Rossmann-like fold. The conservation of this motif across species implies a fundamental role in the protein's function.

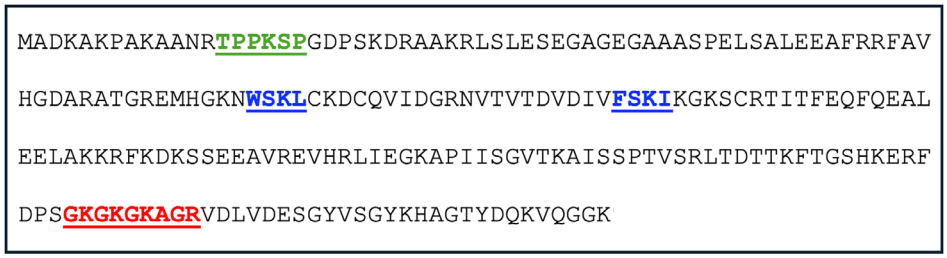

FIGURE 4 | Protein sequence of human TPPP/p25. Presence of specific motifs in human TPPP corresponding to a phosphorylation site (green, TPPKSP) within this segment is also present in tau protein; LC3B-interacting region (LIR) motif (blue, WSKL and FSKI), and a Rossmann motif (red, GKGKGKRGR).

However, the immunoreactivity of TPPP in the neurons remains to be established (Kon et al. 2024). In the adult rat brain, TPPP expression is seen in most regions of the CNS and neuropil (Takahashi et al. 1993). TPPP is expressed in oligodendrocytes and in the specialized membrane of the choroid plexus. In the pre- and post-natal rat brain, TPPP is localized to the perinuclear cytoplasm of myelinating oligodendrocytes from embryonic day 20 (E20) that was verified from the cellular co-localization with 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNP) (Lee et al. 2005). Oligodendrocyte progenitor cells and pre-myelinating oligodendrocytes identified by the expression of NG2 proteoglycan and CD9, respectively, did not show co-localization with TPPP. TPPP co-localized with beta (IV)-tubulin from post-natal day 10 (P10) in developing, myelinating oligodendrocytes. TPPP is expressed in the supraoptic nucleus (Lindersson et al. 2005), hippocampal neurons, and detected in synaptic membrane and synaptic vesicle preparations (Frykman et al. 2012). In the mouse brain, TPPP mRNA expression is high in oligodendrocytes and low in astrocytes, microglia, and neurons (Goldbaum et al. 2008; Fu et al. 2019).

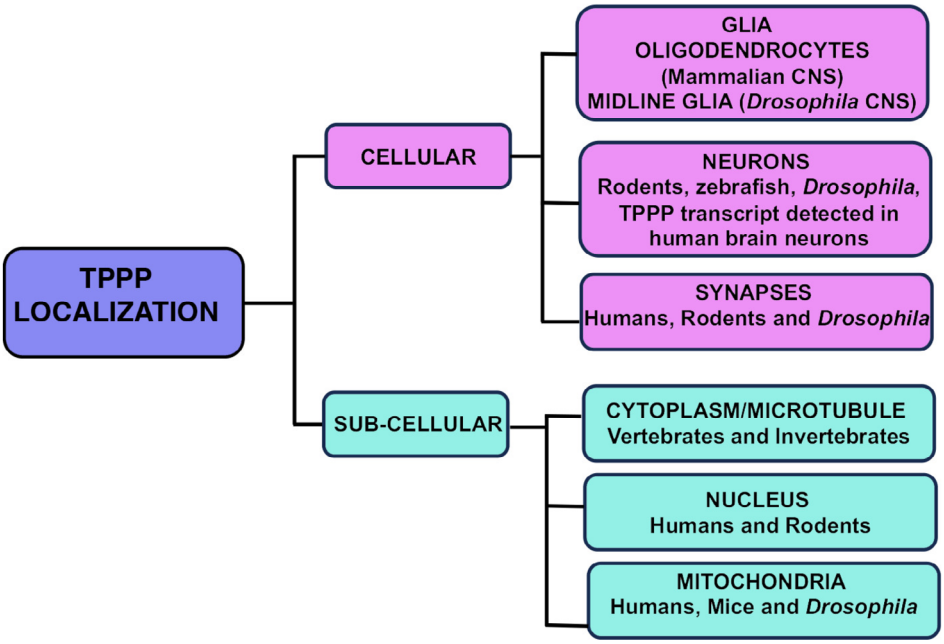

FIGURE 5 | TPPP localization in the nervous system across species. A schematic illustration of the various cell types and subcellular compartments that TPPP localizes to.

TPPP is detected as puncta along oligodendrocyte processes coinciding with Golgi outposts that act as sites of local microtubule nucleation and were also reported in *Drosophila* (Ori-McKenney et al. 2012) and in mammalian neuronal dendrites (Horton et al. 2005; Quassollo et al. 2015). TPPP is also detected in the neuropil and synapses (Frykman et al. 2012; Tripon et al. 2018; Moritz et al. 2019). TPPP is expressed in the zinc-rich retina and its localization both in the mice and human eyes was observed in the postsynaptic nerve terminals (Tripon et al. 2018).

Expression of the *Drosophila* TPPP, named Ringer (Mino et al. 2016), is dynamically regulated in the embryonic CNS with initial expression in pioneer midline neurons and axons that initiate the formation of intersegmental connections (Kuzina et al. 2011). However, during later stages of embryonic ventral nerve cord development, Ringer expression became stronger in the midline glia. Midline glia are akin to the vertebrate oligodendrocytes as they perform crucial neuron–glial interactions and ensheathment of commissural axons in the CNS (Jacobs 2000; Banerjee et al. 2017). Besides the embryonic nervous system, Ringer is expressed in the larval sensory neurons (Vargas et al. 2020) and localizes to the larval presynaptic neuromuscular junction terminals (Shi et al. 2019). It is also abundantly expressed in all neurons of the adult fly brain (Xie et al. 2021). Similar to the fly TPPP/Ringer, expression of TPPP/TPPP-like proteins was observed in the sensory trigeminal neurons, spinal neurons including RB neurons, and primary motor neurons in wild-type zebrafish embryos (Aoki et al. 2014). Thus, TPPP across species is abundantly expressed in the nervous system and is enriched in oligodendrocytes but also has neuronal and synaptic expression indicating its capability of performing diverse cellular functions.

3.2 | SubCellular Localization of TPPP

In healthy human brain, TPPP is localized in the cytoplasmic compartment of the oligodendrocytes, including perinuclear cytoplasm and peripheral processes in the white matter, and in a subset of oligodendrocyte nuclei. Immunoelectron microscopy studies have further confirmed subcellular TPPP localization in the nucleus and mitochondrial membranes of healthy oligodendrocytes. In addition, biochemical subcellular fractionation and immunoblotting analyses also supported the nuclear and mitochondrial presence of mammalian TPPP (Ota et al. 2014). Significant TPPP transcript expression was recently reported both in the nucleus and cytoplasm of neurons at the pontine base of healthy human brains (Kon et al. 2024). In invertebrates, such as *Drosophila*, endogenous TPPP/Ringer is expressed in the cytoplasm and the microtubular network, both in the midline glia/glial processes as well as in the neuronal and synaptic microtubules (Mino et al. 2016; Shi et al. 2019; Vargas et al. 2020). In the adult fly brain, TPPP/Ringer localizes to the neuronal mitochondria, as revealed from immunohistochemical and biochemical subcellular fractionation experiments (Xie et al. 2021). Thus, the diverse cellular and subcellular localizations of TPPP are a major contributing factor toward its functional diversity.

4 | Diverse TPPP Functions

The most well-established in vitro and in vivo physiological function of TPPP is similar to that of the MAPs (Hlavanda et al. 2002; Tirian et al. 2003; Lehotzky et al. 2004; Shi et al. 2019). However, besides its microtubular functions, TPPP displays remarkable functional diversity as it plays multiple crucial roles at the cellular and molecular levels that are described below and summarized in Figure 6.

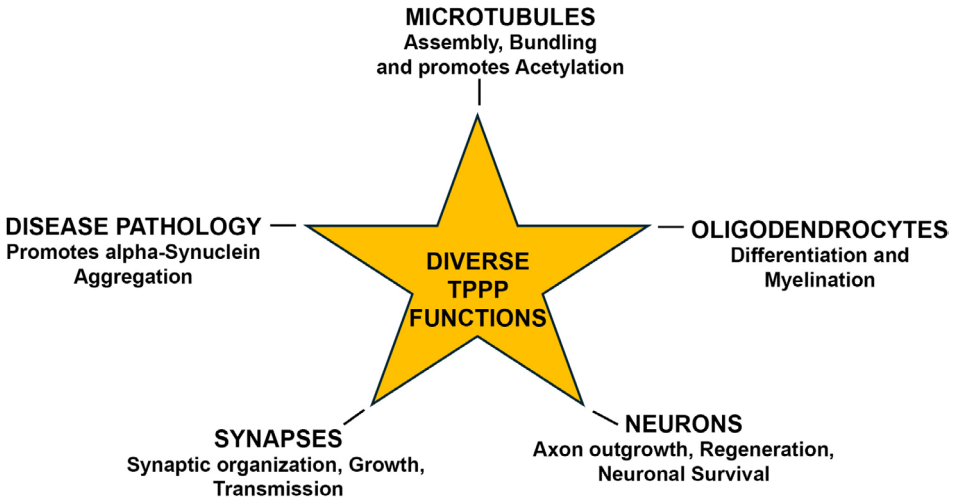

FIGURE 6 | Diverse functions performed by TPPP. TPPP exhibits a broad range of functions in the nervous system across species. Under normal physiological conditions, it is involved in microtubule bundling/polymerization and stability, oligodendrocyte differentiation/myelination, neuronal survival, axon growth/regeneration, and synaptic organization and growth. TPPP is also pro-aggregatory and promotes α-Synuclein aggregation under pathological conditions.

4.1 | Microtubule-Associated Functions

Microtubules, one of the major constituents of the cytoskeleton, are composed of α- and β-tubulin dimers, whose stability is ensured by MAPs (Goodson and Jonasson 2018; Bodakuntla et al. 2019). TPPP binds to the tubulin/microtubular network under in vitro and in vivo conditions (Hlavanda et al. 2002; Tirian et al. 2003; Lehotzky et al. 2004; Vargas et al. 2020). TPPP induces microtubule assembly, and under different circumstances, aberrant forms, such as double-walled microtubules and aggregates, are also formed as visualized by electron and atomic force microscopy (Hlavanda et al. 2002). TPPP displays extensive microtubule bundling activity both in vitro and in vivo, which is one of its physiological functions (Tirian et al. 2003; Lehotzky et al. 2004, 2010; Zotter et al. 2011; Olah et al. 2013; Mino et al. 2016). The bundling activity of TPPP results in the stabilization of the microtubular network (Hlavanda et al. 2002; Vincze et al. 2006; Tokesi et al. 2010) and protects the microtubules against depolymerizing agents (Hlavanda et al. 2002; Lehotzky et al. 2004; Mino et al. 2016). TPPP is capable of dimerization stabilized by disulfide bridges (Olah et al. 2012) which is crucial for its bundling and cross-linking activities (DeBonis et al. 2015; Olah et al. 2017b).

In addition to microtubule bundling activity, TPPP is implicated in regulating the levels of acetylated tubulin (Tokesi et al. 2010; Szabo et al. 2017). Acetylation of microtubules is an evolutionarily conserved posttranslational modification and reflects the stability of the microtubular cytoskeleton (Janke and Montagnac 2017; Eshun-Wilson et al. 2019). Overexpression of TPPP in stable cell lines and in vivo resulted in increased acetylated tubulin levels, while knockdown or loss of TPPP results in decreased levels of acetylated tubulin (Acevedo et al. 2007; Shi et al. 2019). TPPP binds to histone deacetylase 6 (HDAC6) and inhibits its deacetylase activity (Tokesi et al. 2010). TPPP also influences the microtubule dynamics by decreasing the growth velocity of the microtubule plus ends and affects cell motility, further suggesting that TPPP can influence microtubule organization in multiple ways. Thus, TPPP regulates the dynamics and stability of the microtubule network both in vitro and in vivo across species through its roles in promoting microtubule assembly, cross-linking, and enhancing acetylation.

4.2 | TPPP in Oligodendrocyte Differentiation, Myelination, and Behavior Regulation

Using rat primary oligodendrocyte cells and a glial CG-4 cell line, TPPP was found to be significantly upregulated during oligodendrocyte differentiation, indicating its essential role in this process (Lehotzky et al. 2010). This upregulation might be linked to microtubule rearrangement during process elongation prior to the onset of myelination. In vitro studies have also shown that the reduction of TPPP levels in mammalian oligodendrocytes impairs differentiation and process extension, further suggesting its role in cellular process growth during development. Studies from *TPPP* knockout mice oligodendrocytes showed aberrant microtubule branching, mixed microtubule polarity, and hypomyelination with shorter myelin sheaths. Behaviorally, *TPPP* knockout mice displayed motor coordination defects (Fu et al. 2019), lack of fear responses, and revealed deficits in fear conditioning, suggestive of a possible short-term memory deficit in these mice (Nguyen et al. 2020). Together, these studies implicate a role for TPPP in CNS myelination, myelin maintenance, and behavior regulation in mice.

4.3 | TPPP and Aggregation Promoting Properties

In oligodendrocytes, TPPP has been shown to be a potent stimulator of α-Synuclein (α-Syn) aggregation both in vivo and in vitro (Lindersson et al. 2005; Hasegawa et al. 2010; Ejlerskov et al. 2013; Mavroeidi et al. 2019, 2022). There was increased α-Syn aggregation and cell death in oligodendroglial KG1C

cell lines when TPPP was expressed, which was partly rescued by SIRT2, a tubulin deacetylase (Hasegawa et al. 2010). Inhibition of SIRT2 by TPPP has been demonstrated; however, the TPPP-associated microtubule ultrastructure appeared to be resistant to SIRT2 activity (Szabo et al. 2017). The structural and functional effects of TPPP on SIRT2 are thought to provide a refinement of the regulation of microtubule dynamics and stability.

The disordered structure of TPPP contributes to its functional diversity. TPPP displays a range of both physiological and pathological functions by interacting with distinct partners. Using human recombinant TPPP, Tokesi et al. (2014) demonstrated the role of the disordered N- and C-termini straddling a middle flexible segment in the distinct functions of TPPP and identified the binding motifs responsible for its associations with tubulin and α-Syn, believed to be its physiological and pathological interacting partners, respectively. Truncation of the disordered termini of TPPP altered the folding state of the middle segment that had functional consequences related to its binding to tubulin, microtubule assembly promoting, and microtubule bundling activities. Double truncation of N- and C-terminal domains of TPPP diminished its binding to tubulin/microtubules and erased the tubulin polymerization/microtubule bundling activities of TPPP, thus highlighting a role of the disordered termini in its physiological function. Interestingly, the interaction of TPPP with α-Syn and its aggregation promoting abilities were unaffected by these truncations, indicating that the α-Syn binding motif was localized within the middle segment of TPPP (Tokesi et al. 2014). These structure/function analyses suggest that TPPP has unique domains that interact with specific proteins and that this structural diversity may also underlie tissue- or cell-specific pathologies based on the co-expression of these interacting proteins.

4.4 | TPPP and Neuronal Functions

TPPP's neuronal functions were largely identified through studies using *Drosophila*, where it is abundant in neurons throughout development and in the adult brain. The *Drosophila* TPPP/Ringer functions in proper neuronal and glial cell placement, and in axon targeting in the embryonic nervous system (Mino et al. 2016). Ultrastructural examination of *Ringer* mutants revealed defective axonal microtubule morphology, organization, and integrity (Mino et al. 2016; Shi et al. 2019). Ringer is essential for axon outgrowth and axon regeneration (Mino et al. 2016; Vargas et al. 2020). Zebrafish TPPP/TPPP-like protein also performs similar functions as the fly TPPP/Ringer in regulating sensory axon growth in zebrafish embryos (Aoki et al. 2014; Orosz 2015) further demonstrating functional conservation of TPPPs across species. In vivo phenotypic and functional characterization of Ringer showed that Ringer regulates synaptic growth, cytoarchitecture, and synaptic transmission (Shi et al. 2019). Studies from the mouse and human retina also implicated a role of vertebrate TPPP in the organization of synaptic connections and visual integration in vertebrates (Tripon et al. 2018).

Ringer functions in the adult brain neurons and is implicated in neuronal survival (Xie et al. 2021). Adult *Ringer* mutants exhibit progressive locomotor defects, shortened lifespan, and neurodegeneration, including dopaminergic neuron loss, resembling key features of Parkinson's disease (PD). These mutants provide a valuable fly model for studying the genetic and molecular basis of PD and may provide insights into the role of human TPPP in PD. *Ringer* mutants also display mitochondrial structural damage and dysfunction, including higher superoxide levels indicative of increased oxidative stress, reduced mitochondrial membrane potential, and ATP levels (Xie et al. 2021). Uncovering these novel mitochondrial functions of the fly TPPP/Ringer in maintaining mitochondrial bioenergetics will unravel the crucial role of TPPP in neuronal survival.

4.5 | TPPP Phosphorylation

In vitro phosphorylation of wild type and a truncated form of human recombinant TPPP was detected by extracellular signal-regulated kinase 2 (ERK2), cyclin-dependent kinase 5 (Cdk5), and cAMP-dependent protein kinase (PKA). Several phosphorylation sites by these kinases were identified by mass spectrometry (Hlavanda et al. 2007). Three N-terminal sites were also phosphorylated in vivo in TPPP isolated from bovine brain. The phosphorylation of TPPP by ERK2 and Cdk5 perturbed the structural alterations induced by the interaction between TPPP and tubulin and resulted in the loss of microtubule-assembling function of TPPP. A similar effect was observed upon the phosphorylation of TPPP by LIM kinase 1 (Acevedo et al. 2007). The rho-associated coiled coil kinase also phosphorylates TPPP on the serine residues; however, it did not affect the tubulin polymerization-promoting activity of TPPP but inhibited its interaction with HDAC6, resulting in reduced microtubule acetylation (Schofield et al. 2012, 2013). Therefore, the phosphorylation of distinct sites of TPPP by different kinases likely plays an important role in the functional diversity of TPPP. What consequences the phosphorylation states of TPPP would have in the context of human health and disease remains to be investigated.

5 | TPPP-Interacting Proteins

Among additional factors that may contribute toward the functional diversity of TPPP is the wide range of proteins that it interacts with under both physiological and pathological conditions. These include MAPs, cytosolic proteins, various kinases, Ca^{2+}-binding proteins, signaling proteins as well as disease relevant proteins such as prion proteins, α-Syn, amyloid-beta (Aβ), and MBP (summarized in Table 1).

TPPP interacting proteins that influence the microtubular network are tubulin, MAP1B/Futsch, and the tubulin deacetylase, HDAC6 (Tokesi et al. 2010; Olah et al. 2019; Shi et al. 2019; Vargas et al. 2020). TPPP was identified as a novel S100A2-binding protein (Doi et al. 2021) that belongs to the EF hand-type Ca^{2+}-binding protein family and plays a role in various intracellular Ca^{2+}-signaling and other extracellular activities (Heizmann et al. 2002; Donato et al. 2013). The cytosolic protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH), widely considered a housekeeping protein, was identified as an interacting partner of TPPP and was found to be co-enriched in the Lewy bodies under pathological conditions in the human brain (Olah et al. 2006).

TABLE 1 | TPPP interacting proteins.

Protein name	Nature of protein	References
Futsch/microtubule associated protein (MAP1B)	MAP	Shi et al. (2019)
Tubulin	Cytoskeletal	Hlavanda et al. (2002), Shi et al. (2019), Vargas et al. (2020)
Histone Deacetylase (HDAC6)	Tubulin Deacetylase	Tokesi et al. (2010), Olah et al. (2019)
Dynein light-chain DYNLL/LC8	MAP	Olah et al. (2019)
Sirtuin 2 (SIRT2)	Cytoplasmic	Szabo et al. (2017)
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Cytoplasmic	Olah et al. (2006)
S100A12	Cytoplasmic/Ca^{2+} binding	Doi et al. (2021)
Prion protein (PrP)	Cytoplasmic	Zhou et al. (2011)
(Myelin Basic Protein) MBP	Cytoplasmic	Song et al. (2007), Hasegawa et al. (2010)
Parkinsonism-associated deglycase 7/DJ-1	Cytoplasmic, mitochondrial translocation under oxidative stress	Olah et al. (2021)
α-Synuclein	Cytoplasmic	Szunyogh et al. (2015), Hasegawa et al. (2010)
γ-secretase	Late endosome/lysosome	Frykman et al. (2012)
Amyloid β	Extracellular/mitochondrial-associated membrane	Olah et al. (2011), JBC
Complexin	Cytoplasmic/presynaptic terminals	Nelson et al. (2004)
MAP 1A/1B light-chain 3B (LC3B)	Cytoplasmic	Lehotzky et al. (2021)
Lim domain kinase 1 and 2 (LIMK1 and LIMK2)	Serine/Threonine Kinase	Acevedo et al. (2007), Heng et al. (2012)
Extracellular signal-regulated kinase 2 (ERK2)	Serine/Threonine Kinase	Hlavanda et al. (2007)
Glycogen synthase kinase 3 (GSK-3)	Serine/Threonine Kinase	Martin et al. (2002)
Cyclin dependent kinase 5 (Cdk5)	Serine/Threonine Kinase	Martin et al. (2002)
Protein kinase A (PKA)	Serine/Threonine Kinase	Martin et al. (2002)
Yin Yang 1	Transcription factor	Chen et al. (2019)

TPPP interacts with various disease-associated proteins. For example, TPPP forms a molecular complex with the cytosolic prion proteins (PrP) and influences the aggregation and fibrillization of PrP (Zhou et al. 2011). Interactions between TPPP and α-Syn were shown using human recombinant proteins (Szunyogh et al. 2015) and cell lines such as oligodendroglial KG1C (Hasegawa et al. 2010). TPPP also binds to myelin basic protein (MBP), a protein implicated in the pathogenesis of Multiple Sclerosis (MS) (Song et al. 2007; Hasegawa et al. 2010; Martinsen and Kursula 2022). TPPP interacts with DJ-1, a highly conserved protein involved in oxidative stress response, which is implicated in human PD (Bonifati et al. 2003). TPPP was identified as an interacting partner of γ-secretase from rat brain synaptic membrane and synaptic vesicle preparations and was found to regulate Aβ levels (Frykman et al. 2012).

Among various kinases, direct interaction between TPPP and ERK2 was demonstrated using affinity binding experiments (Hlavanda et al. 2007). TPPP also interacted directly with the PDZ domain of LIMK1 (Acevedo et al. 2007). TPPP interactions were also demonstrated with GSK-3, Cdk5, and Protein kinase A (Martin et al. 2002). These findings indicate that TPPP may potentially function in a variety of cellular processes, many of which are yet to be characterized, including metabolism, signaling, synaptic plasticity, and gene transcription.

In vivo interactions between Complexin and TPPP were reported from studies on spatial learning and memory consolidation performed in the rat hippocampus (Nelson et al. 2004). TPPP's involvement in the memory-specific changes that involve synapse formation, structural changes, and reorganization of growth cones is further suggestive of a role of TPPP in synaptic structural reorganization. Interactions between TPPP and the well-known autophagy protein, LC3B, were reported recently (Lehotzky et al. 2021). The majority of the proteins degraded by selective autophagy can be recognized by the presence of short, conserved sequence motifs

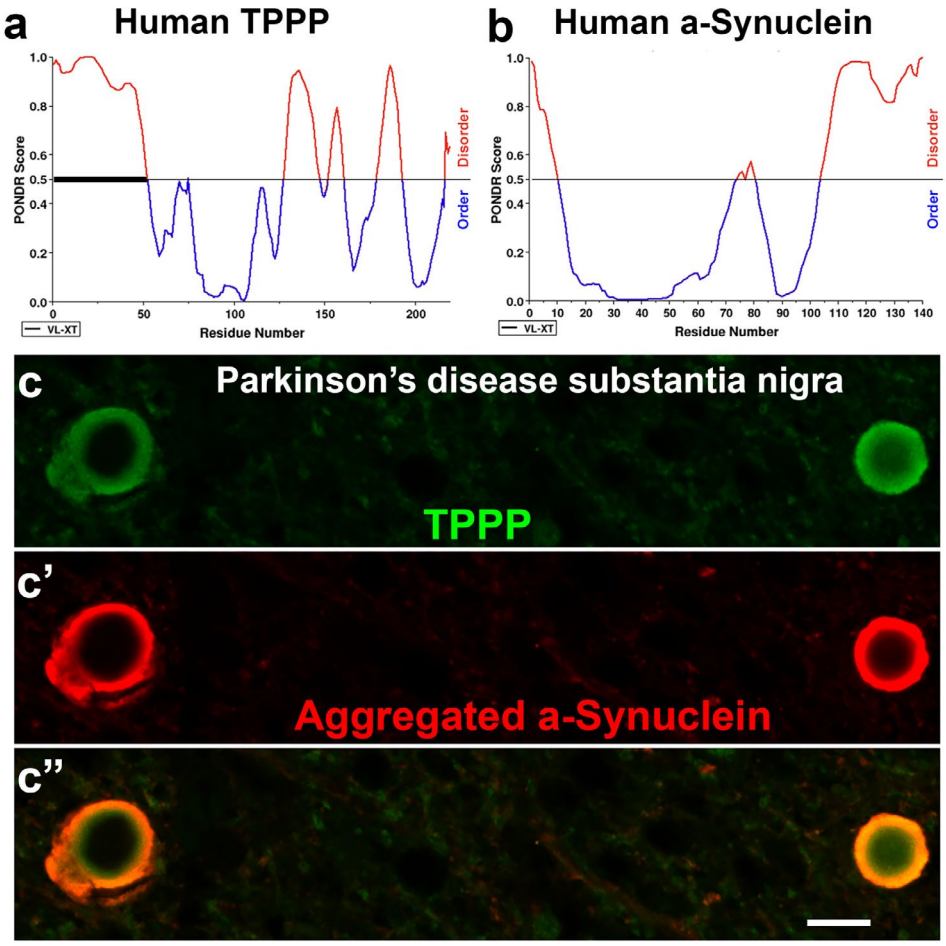

FIGURE 7 | Disorder prediction in human TPPP and α-Synuclein and their co-aggregation in Lewy bodies of Parkinson's disease. (a, b) The predicted disorder in the full-length human TPPP (a) and α-Synuclein (b) by PONDR (Predictor of Natural Disordered Region; Orosz et al. 2004). Protein sequences were obtained from NCBI database. The accession number of human TPPP is O94811.1 and human α-Synuclein is P37840.1. (c–c″) Confocal images from a Parkinson's disease patient substantia nigra brain region labeled with human anti-TPPP (green, c, c″) and anti-aggregated α-Synuclein (red, c′, c″) antibodies show co-aggregation of TPPP and α-Synuclein in Lewy bodies. Anti-TPPP (Kovacs et al. 2004) and anti-aggregated α-Synuclein (Abcam, ab209538) were used at 1:800 and 1:400 dilutions, respectively. Secondary antibodies conjugated to Alexa-488 and Alexa-568 (Invitrogen) were used at 1:400 dilution. Scale bar in c–c″ = 10 μm.

known as LC3-interacting regions (LIR) (Birgisdottir et al. 2013), which can be found within the sequence of human TPPP (Figure 4, Lehotzky et al. 2021). This interaction may have important implications in the clearance of toxic protein assemblies in neurodegenerative diseases involving TPPP.

6 | Clinical Relevance of TPPP

Human TPPP has immense clinical relevance, which further underscores its significance. Aberrant expression or dysfunction of TPPP has been implicated in various pathological conditions, including various nervous system disorders, as highlighted below.

6.1 | TPPP and α-Synucleinopathies

TPPP is associated with human α-synucleinopathies, a group of neurodegenerative disorders that include PD, Lewy body dementia (LBD), also considered an Alzheimer's disease-related dementia (ADRD), and multiple system atrophy (MSA) (Spillantini et al. 1997; Gai et al. 1998). The common hallmark of these disorders is the development of α-Syn aggregates in the form of Lewy body inclusions in neuronal soma and Lewy neurites in axons as seen in PD and LBD (Goedert et al. 2013; Kalia and Lang 2015; Yang et al. 2022), and oligodendroglial inclusions in MSA referred to as Papp-Lantos inclusions (Papp et al. 1989; Wakabayashi et al. 2024). TPPP co-localizes with α-Syn in the Lewy bodies, Lewy neurites, and glial cytoplasmic inclusions in these disorders (Kovacs et al. 2004).

Both TPPP and α-Syn are IDPs that display high conformational plasticity. A neural network-based algorithm, Predictor of Natural Disordered Regions (PONDR), that predicts disordered and ordered regions of proteins shows the disordered regions of human TPPP and α-Syn (red, Figure 7a,b, respectively; Orosz et al. 2004). The N-terminal region of TPPP is disordered. Although the protein sequence of TPPP is very different from that of α-Syn, there still exist similarities in their unstructured segments interrupted by stabilization centers, phosphorylation, and tubulin binding motifs (Orosz et al. 2004). TPPP was also determined to be an IDP using NMR spectroscopy (Kovacs

et al. 2004; Orosz et al. 2004). Consistent with previous studies, immunofluorescence labeling and confocal microscopy demonstrate convincingly that TPPP (Figure 7c,c″) is enriched in α-Syn positive Lewy bodies (Figure 7c′,c″) of PD patients from substantia nigra (Figure 7c–c″).

Although TPPP has been demonstrated as a pro-aggregatory protein promoting α-Syn aggregation, interestingly, there are proteins identified that can counter this effect. One such protein identified recently is DJ-1 from studies using human cells that revealed antiaggregation effects of DJ-1 on the toxic assembly of TPPP and α-Syn (Olah et al. 2021). This was attributed to an increase in the proteolytic degradation of α-Syn/TPPP complex. DJ-1 may also modulate the α-Syn/TPPP aggregation process by binding directly to the proteins as has been demonstrated in studies using a human cell line (Olah et al. 2021). These data suggest a protective effect of DJ-1 against toxic α-Syn assemblies and have therapeutic potential. The identification of more such proteins that function at the pathological interface promoting the disassembly of α-Syn/TPPP and have neuroprotective effects may serve as therapeutic targets in the development of new treatments for α-synucleinopathies.

Clearance of damaged organelles, misfolded/aggregated proteins and intracellular pathogens by autophagy and the ubiquitin-proteasome system underlie the major protein quality control mechanisms in eukaryotic cells and are implicated both in health and disease (Kocaturk and Gozuacik 2018). TPPP was shown to stimulate secretion of α-Syn aggregates from neurons by blocking autophagic pathways (Ejlerskov et al. 2013). This could contribute to noncell autonomous activation of microglia and astrocytes and in turn unleash a strong neuroinflammatory response and neuronal loss. Association of α-Syn with TPPP after their uptake from the medium by human cells inhibited their elimination by autophagy and the ubiquitin-proteasome system. This was achieved by TPPP's hindering of the autophagy maturation at the stage of LC3B-SQSTM1/p62-derived autophagosome formation and its fusion with the lysosome (Lehotzky et al. 2021). Evidence of TPPP-overexpressing oligodendroglial cells taking up human preformed α-Syn fibrils was also shown to form insoluble, highly aggregated, pathological assemblies, and autophagy-mediated clearance was also demonstrated (Mavroeidi et al. 2019, 2022). The co-aggregation of TPPP with α-Syn frequently results in more toxic α-Syn strains or species that are resistant to degradation (Ferreira et al. 2021). Therefore, a better understanding of degradation pathways becomes crucial. In recent studies, using the nerve growth factor-differentiated catecholaminergic PC12 neuronal cell line, TPPP was shown to aggregate α-Syn and impose a partial autophagosome-lysosome block to replicate aspects of lysosomal deficiency commonly seen in neurodegenerative diseases (Borland et al. 2022).

Human MSA patients show dysregulation of MBP and TPPP (Song et al. 2007; Wenning et al. 2008). In these patient brains, TPPP relocates within oligodendroglial compartments away from the myelin sheath toward the cell body and shows enlarged oligodendrocytes (Song et al. 2007). TPPP accumulation within affected oligodendrocytes in MSA is considered an early detectable change with potential relevance to early myelin disruption. Co-localization of TPPP with MBP is markedly reduced in MSA brain in addition to altered distribution of TPPP (Song et al. 2007). Together, these changes suggest a sequence of pathogenic events in which the normal cellular function of TPPP in myelin is affected with decreased stability of MBP and increased aggregation of α-Syn with disease progression (Song et al. 2007; Wenning et al. 2008). While under physiological conditions, TPPP is expressed in the oligodendroglial nucleus, cytoplasm, and myelin (Acevedo et al. 2007; Hoftberger et al. 2010; Kovacs et al. 2004; Lindersson et al. 2005; Olah et al. 2006; Skjoerringe et al. 2006), TPPP redistributes from the nucleus and myelin sheath to an abnormally expanded perinuclear cytoplasm in pathological conditions (Ota et al. 2014; Song et al. 2007). While the significance of this TPPP redistribution, its contribution toward the progress of the pathology or its underlying mechanisms are still unclear, it is not uncommon, however, for proteins to redistribute/mislocalize in different subcellular compartments in neurodegenerative diseases (Suk and Rousseaux 2020), which is a reflection of the physiological changes occurring in specific cell types during pathogenesis.

6.2 | TPPP and Alzheimer's Disease

Co-occurrence of α-Syn with Aβ and tau in human brain inclusions has long been reported (Moussaud et al. 2014), suggesting the existence of mixed pathologies and crosstalk between proteinopathies, thus posing significant challenges in the accurate disease diagnosis and treatment. While TPPP is well documented in cases of α-synucleinopathies to coaggregate with α-Syn, what role it plays in Alzheimer's disease (AD) and ADRD neuropathology is not well understood. TPPP was identified as an interacting partner of the soluble Aβ oligomers, which are major risk factors for AD (Olah et al. 2011) and has also been shown to partially colocalize with Aβ in diffuse LBD with AD (Kovacs et al. 2004). Immunopositivity of TPPP was also documented in postmortem brain tissue of AD patients at the pretangles, and punctate TPPP localization was observed in the neuronal cytoplasm in areas with abundant tau pathology (Kovacs et al. 2004). TPPP was also found to accumulate in intraneuronal granules and fibrous structures in the hippocampus from human AD subjects (Frykman et al. 2012). Interestingly, a phosphorylation site motif (TPPKSP, Figure 4) is present in the unstructured part of both TPPP and tau proteins (Orosz et al. 2004). This sequence was described as a Cdk5 phosphorylation site in tau and was shown to be phosphorylated by Cdk5 in TPPP as well in vitro (Takahashi et al. 1991). It could be speculated that the phosphorylation of this site in TPPP may also have physiological/pathological significance. Thus, TPPP could potentially be involved in multiple pathological protein–protein interactions leading to protein aggregations characteristic of several neurodegenerative proteinopathies.

6.3 | TPPP and Multiple Sclerosis

The studies to date implicate a crucial role of TPPP in other neurological disorders, such as multiple sclerosis (MS). MS is a chronic inflammatory demyelinating disease of the CNS that has variable extents of remyelination (Filippi et al. 2018). Using monoclonal anti-TPPP antibodies, oligodendrocytes from different subtypes and disease stages of MS patients, including acute, primary progressive, secondary progressive, and relapsing

remitting MS, together with controls, were analyzed along with degenerative changes (Hoftberger et al. 2010). In these studies, demyelinated lesions displayed loss of TPPP-positive oligodendrocytes within the plaques. During remyelination, TPPP was first expressed in the oligodendrocyte cytoplasm, later becoming positive in the myelin sheaths. In addition, increased numbers of TPPP immunoreactive oligodendrocytes in the normal appearing white matter were seen in MS patients. The cytoplasmic area of TPPP immunopositivity in the oligodendrocytes was also significantly higher in the peri-plaque white matter as in other lesions. Interestingly, the cytoplasmic area of TPPP immunoreactivity correlated inversely with the duration of the disease. In addition, significantly increased levels of TPPP were also detected in the cerebrospinal fluid of MS patients compared to non-MS controls (Vincze et al. 2011). Impaired oligodendrocyte differentiation, migration, and activation capacity were also observed in later disease stages of MS (Vincze et al. 2011). Given that TPPP reduction impacts the differentiation of oligodendrocyte progenitors and the development of myelin projections required for the myelination/remyelination process (Lehotzky et al. 2010), the demyelination and/or remyelination deficits in MS could be related to the reorganization and stabilization of the oligodendroglial microtubular network required for ensheathment of axons. Whether the altered levels and distribution of TPPP is a primary consequence of the disease or a secondary consequence of the impacted oligodendrocytes and possibly other myelin genes needs further investigations.

7 | Conclusions and Future Areas of Study

As summarized in this article, TPPP plays multiple pivotal roles in cellular processes in health and disease. Several factors that contribute to the functional diversity of TPPP include its diverse cellular and sub-cellular localization, disordered structure, wide range of binding/interacting partners, posttranslational modifications, and presence of distinct sequence motifs in the TPPP protein. Despite the progress made toward understanding TPPP biology since its initial discovery, several key questions outlined below, particularly in the context of neurodegenerative disorders, remain unanswered and could serve as potential areas for future study to uncover the mechanisms underlying the associated pathologies.

What role does TPPP phosphorylation play in neurodegenerative disorders? Posttranslational modifications are known to contribute to neurodegeneration (Iqbal et al. 2009). The phosphorylation and other posttranslational modifications of TPPP may alter its activity or associations with other proteins in neurological disorders. Thus, understanding these molecular interactions is key to developing therapeutic targets for TPPP-related nervous system diseases.

Are TPPP's mitochondrial functions independent of its microtubular functions? Mitochondria–microtubule interactions are crucial for mitochondrial morphology, transport, and function. TPPP, a unique MAP, also influences mitochondrial structure and function. Given that both microtubules and mitochondria are vital for neuronal survival and their dysfunction is linked to neurodegenerative disorders (Johri and Beal 2012; Sferra et al. 2020), it is important to explore whether TPPP's roles in microtubules and mitochondria are independent or interlinked in neuronal survival and neurodegeneration, and whether these roles could have distinct molecular pathways.

What role does TPPP play in the nucleus? Although studies have reported subcellular nuclear localization of TPPP (Ota et al. 2014; Hoftberger et al. 2010; Acevedo et al. 2007), the specific roles that TPPP plays in the nucleus are an interesting area of future studies. Human TPPP has predicted nuclear localization signals indicating its potential translocation to the nucleus and is speculated to play a role in nucleocytoplasmic transport (Ota et al. 2014). Could the nuclear presence of TPPP have any consequences on the chromatin structure or nucleocytoplasmic transport as both these processes are linked to neurodegenerative diseases including AD, ALS, and FTD (Zhang et al. 2015)?

Given the common crosstalk between proteinopathies, it remains to be elucidated whether TPPP serves as a potential link between various proteinopathies, especially considering its aggregation-promoting properties. A much deeper understanding of TPPP biology will broaden our knowledge of many cellular processes it is involved in, both in healthy and diseased states, and may uncover novel therapeutic targets for related neurological diseases.

Declaration of Transparency

The authors, reviewers and editors affirm that in accordance to the policies set by the *Journal of Neuroscience Research*, this manuscript presents an accurate and transparent account of the study being reported and that all critical details describing the methods and results are present.

Author Contributions

Paloma J. Diaz: investigation, writing – review and editing. **Qian Shi:** formal analysis. **Priscilla Y. McNeish:** investigation, writing – review and editing. **Swati Banerjee:** conceptualization, project administration, resources, supervision, funding acquisition, writing – original draft, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The authors have nothing to report.

References

Acevedo, K., R. Li, P. Soo, et al. 2007. "The Phosphorylation of p25/TPPP by LIM Kinase 1 Inhibits Its Ability to Assemble Microtubules." *Experimental Cell Research* 313: 4091–4106.

Alagundagi, D. B., S. D. Ghate, P. Shetty, P. Gollapalli, P. Shetty, and P. Patil. 2023. "Integrated Molecular-Network Analysis Reveals Infertility-Associated Key Genes and Transcription Factors in the Non-Obstructive Azoospermia." *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 288: 183–190.

Aoki, M., H. Segawa, M. Naito, and H. Okamoto. 2014. "Identification of Possible Downstream Genes Required for the Extension of Peripheral Axons in Primary Sensory Neurons." *Biochemical and Biophysical Research Communications* 445: 357–362.

Banerjee, S., R. E. Mino, E. S. Fisher, and M. A. Bhat. 2017. "A Versatile Genetic Tool to Study Midline Glia Function in the *Drosophila* CNS." *Developmental Biology* 429: 35–43.

Birgisdottir, A. B., T. Lamark, and T. Johansen. 2013. "The LIR Motif—Crucial for Selective Autophagy." *Journal of Cell Science* 126: 3237–3247.

Bodakuntla, S., A. S. Jijumon, C. Villablanca, C. Gonzalez-Billault, and C. Janke. 2019. "Microtubule-Associated Proteins: Structuring the Cytoskeleton." *Trends in Cell Biology* 29: 804–819.

Bonifati, V., P. Rizzu, M. J. van Baren, et al. 2003. "Mutations in the DJ-1 Gene Associated With Autosomal Recessive Early-Onset Parkinsonism." *Science* 299, no. 5604: 256–259. https://doi.org/10.1126/science.1077209.

Borland, H., I. Rasmussen, K. Bjerregaard-Andersen, M. Rasmussen, A. Olsen, and F. Vilhardt. 2022. "Alpha-Synuclein Buildup Is Alleviated via ESCRT-Dependent Endosomal Degradation Brought About by p38MAPK Inhibition in Cells Expressing p25alpha." *Journal of Biological Chemistry* 298, no. 11: 102531. https://doi.org/10.1016/j.jbc.2022.102531.

Chen, Q., C. Yang, L. Chen, et al. 2019. "YY1 Targets Tubulin Polymerisation-Promoting Protein to Inhibit Migration, Invasion and Angiogenesis in Pancreatic Cancer via p38/MAPK and PI3K/AKT Pathways." *British Journal of Cancer* 121: 912–921.

DeBonis, S., E. Neumann, and D. A. Skoufias. 2015. "Self Protein-Protein Interactions Are Involved in TPPP/p25 Mediated Microtubule Bundling." *Scientific Reports* 5: 13242.

Doi, S., N. Fujioka, S. Ohtsuka, et al. 2021. "Regulation of the Tubulin Polymerization-Promoting Protein by ca(2+)/S100 Proteins." *Cell Calcium* 96: 102404.

Donato, R., B. R. Cannon, G. Sorci, et al. 2013. "Functions of S100 Proteins." *Current Molecular Medicine* 13: 24–57.

Ejlerskov, P., I. Rasmussen, T. T. Nielsen, et al. 2013. "Tubulin Polymerization-Promoting Protein (TPPP/p25alpha) Promotes Unconventional Secretion of Alpha-Synuclein Through Exophagy by Impairing Autophagosome-Lysosome Fusion." *Journal of Biological Chemistry* 288: 17313–17335.

Eshun-Wilson, L., R. Zhang, D. Portran, et al. 2019. "Effects of Alpha-Tubulin Acetylation on Microtubule Structure and Stability." *Proceedings of the National Academy of Sciences of the United States of America* 116: 10366–10371.

Ferreira, N., H. Gram, Z. A. Sorrentino, et al. 2021. "Multiple System Atrophy-Associated Oligodendroglial Protein p25alpha Stimulates Formation of Novel Alpha-Synuclein Strain With Enhanced Neurodegenerative Potential." *Acta Neuropathologica* 142: 87–115.

Filippi, M., A. Bar-Or, F. Piehl, et al. 2018. "Multiple Sclerosis." *Nature Reviews Disease Primers* 4: 43.

Frykman, S., Y. Teranishi, J. Y. Hur, et al. 2012. "Identification of Two Novel Synaptic Gamma-Secretase Associated Proteins That Affect Amyloid Beta-Peptide Levels Without Altering Notch Processing." *Neurochemistry International* 61: 108–118.

Fu, M. M., T. S. McAlear, H. Nguyen, et al. 2019. "The Golgi Outpost Protein TPPP Nucleates Microtubules and Is Critical for Myelination." *Cell* 179: 132–146.e14.

Gai, W. P., J. H. Power, P. C. Blumbergs, and W. W. Blessing. 1998. "Multiple-System Atrophy: A New Alpha-Synuclein Disease?" *Lancet* 352: 547–548.

Geremek, M., E. Zietkiewicz, M. Bruinenberg, et al. 2014. "Ciliary Genes Are Down-Regulated in Bronchial Tissue of Primary Ciliary Dyskinesia Patients." *PLoS One* 9: e88216.

Goedert, M., M. G. Spillantini, K. Del Tredici, and H. Braak. 2013. "100 Years of Lewy Pathology." *Nature Reviews. Neurology* 9: 13–24.

Goldbaum, O., P. H. Jensen, and C. Richter-Landsberg. 2008. "The Expression of Tubulin Polymerization Promoting Protein TPPP/p25alpha Is Developmentally Regulated in Cultured Rat Brain Oligodendrocytes and Affected by Proteolytic Stress." *Glia* 56: 1736–1746.

Goodson, H. V., and E. M. Jonasson. 2018. "Microtubules and Microtubule-Associated Proteins." *Cold Spring Harbor Perspectives in Biology* 10, no. 6: a022608. https://doi.org/10.1101/cshperspect.a022608.

Hasegawa, T., T. Baba, M. Kobayashi, et al. 2010. "Role of TPPP/p25 on Alpha-Synuclein-Mediated Oligodendroglial Degeneration and the Protective Effect of SIRT2 Inhibition in a Cellular Model of Multiple System Atrophy." *Neurochemistry International* 57: 857–866.

Heizmann, C. W., G. Fritz, and B. W. Schafer. 2002. "S100 Proteins: Structure, Functions and Pathology." *Frontiers in Bioscience* 7: d1356–d1368.

Heng, Y. W., H. H. Lim, T. Mina, et al. 2012. "TPPP Acts Downstream of RhoA-ROCK-LIMK2 to Regulate Astral Microtubule Organization and Spindle Orientation." *Journal of Cell Science* 125, no. Pt 6: 1579–1590.

Hlavanda, E., E. Klement, E. Kokai, et al. 2007. "Phosphorylation Blocks the Activity of Tubulin Polymerization-Promoting Protein (TPPP): Identification of Sites Targeted by Different Kinases." *Journal of Biological Chemistry* 282: 29531–29539.

Hlavanda, E., J. Kovacs, J. Olah, F. Orosz, K. F. Medzihradszky, and J. Ovadi. 2002. "Brain-Specific p25 Protein Binds to Tubulin and Microtubules and Induces Aberrant Microtubule Assemblies at Substoichiometric Concentrations." *Biochemistry* 41: 8657–8664.

Hoftberger, R., S. Fink, F. Aboul-Enein, et al. 2010. "Tubulin Polymerization Promoting Protein (TPPP/p25) as a Marker for Oligodendroglial Changes in Multiple Sclerosis." *Glia* 58: 1847–1857.

Horton, A. C., B. Racz, E. E. Monson, A. L. Lin, R. J. Weinberg, and M. D. Ehlers. 2005. "Polarized Secretory Trafficking Directs Cargo for Asymmetric Dendrite Growth and Morphogenesis." *Neuron* 48: 757–771.

Iqbal, K., F. Liu, C. X. Gong, C. Alonso Adel, and I. Grundke-Iqbal. 2009. "Mechanisms of Tau-Induced Neurodegeneration." *Acta Neuropathologica* 118: 53–69.

Jacobs, J. R. 2000. "The Midline Glia of *Drosophila*: A Molecular Genetic Model for the Developmental Functions of Glia." *Progress in Neurobiology* 62: 475–508.

Janke, C., and G. Montagnac. 2017. "Causes and Consequences of Microtubule Acetylation." *Current Biology* 27: R1287–R1292.

Jellinger, K. A., and P. L. Lantos. 2010. "Papp-Lantos Inclusions and the Pathogenesis of Multiple System Atrophy: An Update." *Acta Neuropathologica* 119: 657–667.

Johri, A., and M. F. Beal. 2012. "Mitochondrial Dysfunction in Neurodegenerative Diseases." *Journal of Pharmacology and Experimental Therapeutics* 342: 619–630.

Kalia, L. V., and A. E. Lang. 2015. "Parkinson's Disease." *Lancet* 386: 896–912.

Kocaturk, N. M., and D. Gozuacik. 2018. "Crosstalk Between Mammalian Autophagy and the Ubiquitin-Proteasome System." *Frontiers in Cell and Development Biology* 6: 128.

Kon, T., S. L. Forrest, S. Lee, et al. 2024. "SNCA and TPPP Transcripts Increase in Oligodendroglial Cytoplasmic Inclusions in Multiple System Atrophy." *Neurobiology of Disease* 198: 106551.

Kovacs, G. G., L. Laszlo, J. Kovacs, et al. 2004. "Natively Unfolded Tubulin Polymerization Promoting Protein TPPP/p25 Is a Common Marker of Alpha-Synucleinopathies." *Neurobiology of Disease* 17: 155–162.

Kuzina, I., J. K. Song, and E. Giniger. 2011. "How Notch Establishes Longitudinal Axon Connections Between Successive Segments of the *Drosophila* CNS." *Development* 138: 1839–1849.

Lee, J., M. Gravel, R. Zhang, P. Thibault, and P. E. Braun. 2005. "Process Outgrowth in Oligodendrocytes Is Mediated by CNP, a Novel Microtubule Assembly Myelin Protein." *Journal of Cell Biology* 170: 661–673.

Lehotzky, A., J. Olah, J. T. Fekete, et al. 2021. "Co-Transmission of Alpha-Synuclein and TPPP/p25 Inhibits Their Proteolytic Degradation in Human Cell Models." *Frontiers in Molecular Biosciences* 8: 666026.

Lehotzky, A., L. Tirian, N. Tokesi, et al. 2004. "Dynamic Targeting of Microtubules by TPPP/p25 Affects Cell Survival." *Journal of Cell Science* 117: 6249–6259.

Lehotzky, A., P. Lau, N. Tokesi, N. Muja, L. D. Hudson, and J. Ovadi. 2010. "Tubulin Polymerization-Promoting Protein (TPPP/p25) is Critical for Oligodendrocyte Differentiation." *Glia* 58: 157–168.

Linck, R., X. Fu, J. Lin, et al. 2014. "Insights Into the Structure and Function of Ciliary and Flagellar Doublet Microtubules: Tektins, Ca^{2+}–Binding Proteins, and Stable Protofilaments." *Journal of Biological Chemistry* 289: 17427–17444.

Lindersson, E., D. Lundvig, C. Petersen, et al. 2005. "p25alpha Stimulates Alpha-Synuclein Aggregation and Is Co-Localized With Aggregated Alpha-Synuclein in Alpha-Synucleinopathies." *Journal of Biological Chemistry* 280: 5703–5715.

Liu, M., Z. Hu, L. Qi, et al. 2013. "Scanning of Novel Cancer/Testis Proteins by Human Testis Proteomic Analysis." *Proteomics* 13: 1200–1210.

Liu, Q., G. Tan, N. Levenkova, et al. 2007. "The Proteome of the Mouse Photoreceptor Sensory Cilium Complex." *Molecular & Cellular Proteomics* 6: 1299–1317.

Martin, C. P., J. Vazquez, J. Avila, and F. J. Moreno. 2002. "P24, a Glycogen Synthase Kinase 3 (GSK 3) Inhibitor." *Biochimica et Biophysica Acta* 1586: 113–122.

Martinsen, V., and P. Kursula. 2022. "Multiple Sclerosis and Myelin Basic Protein: Insights Into Protein Disorder and Disease." *Amino Acids* 54: 99–109.

Mavroeidi, P., F. Arvanitaki, A. K. Karakitsou, et al. 2019. "Endogenous Oligodendroglial Alpha-Synuclein and TPPP/p25alpha Orchestrate Alpha-Synuclein Pathology in Experimental Multiple System Atrophy Models." *Acta Neuropathologica* 138: 415–441.

Mavroeidi, P., F. Arvanitaki, M. Vetsi, et al. 2022. "Autophagy Mediates the Clearance of Oligodendroglial SNCA/Alpha-Synuclein and TPPP/p25A in Multiple System Atrophy Models." *Autophagy* 18: 2104–2133.

Medvedev, K. E., L. N. Kinch, R. D. Schaeffer, and N. V. Grishin. 2019. "Functional Analysis of Rossmann-Like Domains Reveals Convergent Evolution of Topology and Reaction Pathways." *PLoS Computational Biology* 15: e1007569.

Medvedev, K. E., L. N. Kinch, R. Dustin Schaeffer, J. Pei, and N. V. Grishin. 2021. "A Fifth of the Protein World: Rossmann-Like Proteins as an Evolutionarily Successful Structural Unit." *Journal of Molecular Biology* 433: 166788.

Mino, R. E., S. L. Rogers, A. L. Risinger, C. Rohena, S. Banerjee, and M. A. Bhat. 2016. "*Drosophila* Ringmaker Regulates Microtubule Stabilization and Axonal Extension During Embryonic Development." *Journal of Cell Science* 129: 3282–3294.

Moritz, C. P., T. Muhlhaus, S. Tenzer, T. Schulenborg, and E. Friauf. 2019. "Poor Transcript-Protein Correlation in the Brain: Negatively Correlating Gene Products Reveal Neuronal Polarity as a Potential Cause." *Journal of Neurochemistry* 149: 582–604.

Moussaud, S., D. R. Jones, E. L. Moussaud-Lamodiere, M. Delenclos, O. A. Ross, and P. J. McLean. 2014. "Alpha-Synuclein and Tau: Teammates in Neurodegeneration?" *Molecular Neurodegeneration* 9, no. 43: 43. https://doi.org/10.1186/1750-1326-9-43.

Nelson, T. J., P. S. Backlund Jr., and D. L. Alkon. 2004. "Hippocampal Protein-Protein Interactions in Spatial Memory." *Hippocampus* 14: 46–57.

Nguyen, H., L. M. Meservey, N. Ishiko-Silveria, M. Zhou, T. T. Huang, and M. M. Fu. 2020. "Fear Deficits in Hypomyelinated Tppp Knock-Out Mice." *eNeuro* 7, no. 5: ENEURO.0170-20.2020. https://doi.org/10.1523/ENEURO.0170-20.2020.

Olah, J., A. Lehotzky, T. Szenasi, and J. Ovadi. 2021. "Anti-Aggregative Effect of the Antioxidant DJ-1 on the TPPP/p25-Derived Pathological Associations of Alpha-Synuclein." *Cells* 10: 2909.

Olah, J., A. Lehotzky, T. Szenasi, T. Berki, and J. Ovadi. 2022. "Modulatory Role of TPPP3 in Microtubule Organization and Its Impact on Alpha-Synuclein Pathology." *Cells* 11: 3025.

Olah, J., A. Zotter, E. Hlavanda, et al. 2012. "Microtubule Assembly-Derived by Dimerization of TPPP/p25. Evaluation of Thermodynamic Parameters for Multiple Equilibrium System From ITC Data." *Biochimica et Biophysica Acta* 1820: 785–794.

Olah, J., N. Tokesi, A. Lehotzky, F. Orosz, and J. Ovadi. 2013. "Moonlighting Microtubule-Associated Proteins: Regulatory Functions by Day and Pathological Functions at Night." *Cytoskeleton (Hoboken)* 70: 677–685.

Olah, J., N. Tokesi, O. Vincze, et al. 2006. "Interaction of TPPP/p25 Protein With Glyceraldehyde-3-Phosphate Dehydrogenase and Their Co-Localization in Lewy Bodies." *FEBS Letters* 580: 5807–5814.

Olah, J., O. Vincze, D. Virok, et al. 2011. "Interactions of Pathological Hallmark Proteins: Tubulin Polymerization Promoting Protein/p25, Beta-Amyloid, and Alpha-Synuclein." *Journal of Biological Chemistry* 286: 34088–34100.

Olah, J., S. Szunyogh, T. Szenasi, et al. 2019. "Interactions Between Two Regulatory Proteins of Microtubule Dynamics, HDAC6, TPPP/p25, and the Hub Protein, DYNLL/LC8." *Biochimica et Biophysica Acta—Molecular Cell Research* 1866: 118556.

Olah, J., T. Szenasi, A. Szabo, et al. 2017a. "Tubulin Binding and Polymerization Promoting Properties of Tubulin Polymerization Promoting Proteins Are Evolutionarily Conserved." *Biochemistry* 56: 1017–1024.

Olah, J., T. Szenasi, S. Szunyogh, A. Szabo, A. Lehotzky, and J. Ovadi. 2017b. "Further Evidence for Microtubule-Independent Dimerization of TPPP/p25." *Scientific Reports* 7: 40594.

Ori-McKenney, K. M., L. Y. Jan, and Y. N. Jan. 2012. "Golgi Outposts Shape Dendrite Morphology by Functioning as Sites of Acentrosomal Microtubule Nucleation in Neurons." *Neuron* 76: 921–930.

Orosz, F. 2009. "Apicortin, a Unique Protein, With a Putative Cytoskeletal Role, Shared Only by Apicomplexan Parasites and the Placozoan *Trichoplax adhaerens*." *Infection, Genetics and Evolution* 9: 1275–1286.

Orosz, F. 2012. "A New Protein Superfamily: TPPP-Like Proteins." *PLoS One* 7: e49276.

Orosz, F. 2015. "On the Tubulin Polymerization Promoting Proteins of Zebrafish." *Biochemical and Biophysical Research Communications* 457: 267–272.

Orosz, F. 2022. "On the TPPP Protein of the Enigmatic Fungus, Olpidium-Correlation Between the Incidence of p25alpha Domain and That of the Eukaryotic Flagellum." *International Journal of Molecular Sciences* 23: 13927.

Orosz, F. 2023. "p25alpha Domain-Containing Proteins of Apicomplexans and Related Taxa." *Microorganisms* 11: 1528.

Orosz, F. 2024. "The Role of Tubulin Polymerization-Promoting Protein2 (TPPP2) in Spermatogenesis: A Narrative Review." *International Journal of Molecular Sciences* 25: 7017.

Orosz, F., and J. Ovadi. 2008. "TPPP Orthologs Are Ciliary Proteins." *FEBS Letters* 582: 3757–3764.

Orosz, F., G. G. Kovacs, A. Lehotzky, J. Olah, O. Vincze, and J. Ovadi. 2004. "TPPP/p25: From Unfolded Protein to Misfolding Disease: Prediction and Experiments." *Biology of the Cell* 96: 701–711.

Ota, K., M. Obayashi, K. Ozaki, et al. 2014. "Relocation of p25alpha/Tubulin Polymerization Promoting Protein From the Nucleus to the Perinuclear Cytoplasm in the Oligodendroglia of Sporadic and COQ2 Mutant Multiple System Atrophy." *Acta Neuropathologica Communications* 2: 136.

Ovadi, J., and F. Orosz. 2009. "An Unstructured Protein With Destructive Potential: TPPP/p25 in Neurodegeneration." *BioEssays* 31: 676–686.

Papp, M. I., J. E. Kahn, and P. L. Lantos. 1989. "Glial Cytoplasmic Inclusions in the CNS of Patients With Multiple System Atrophy (Striatonigral Degeneration, Olivopontocerebellar Atrophy and Shy-Drager Syndrome)." *Journal of the Neurological Sciences* 94: 79–100.

Quassollo, G., J. Wojnacki, D. A. Salas, et al. 2015. "A RhoA Signaling Pathway Regulates Dendritic Golgi Outpost Formation." *Current Biology* 25: 971–982.

Schofield, A. V., C. Gamell, and O. Bernard. 2013. "Tubulin Polymerization Promoting Protein 1 (TPPP1) Increases Beta-Catenin Expression Through Inhibition of HDAC6 Activity in U2OS Osteosarcoma Cells." *Biochemical and Biophysical Research Communications* 436: 571–577.

Schofield, A. V., R. Steel, and O. Bernard. 2012. "Rho-Associated Coiled-Coil Kinase (ROCK) Protein Controls Microtubule Dynamics in a Novel Signaling Pathway That Regulates Cell Migration." *Journal of Biological Chemistry* 287: 43620–43629.

Seki, N., A. Hattori, S. Sugano, et al. 1999. "A Novel Human Gene Whose Product Shares Significant Homology With the Bovine Brain-Specific Protein p25 on Chromosome 5p15.3." *Journal of Human Genetics* 44: 121–122.

Sferra, A., F. Nicita, and E. Bertini. 2020. "Microtubule Dysfunction: A Common Feature of Neurodegenerative Diseases." *International Journal of Molecular Sciences* 21: 7354.

Shi, Q., Y. Q. Lin, A. Saliba, J. Xie, G. G. Neely, and S. Banerjee. 2019. "Tubulin Polymerization Promoting Protein, Ringmaker, and MAP1B Homolog Futsch Coordinate Microtubule Organization and Synaptic Growth." *Frontiers in Cellular Neuroscience* 13: 192.

Skjoerringe, T., D. M. Lundvig, P. H. Jensen, and T. Moos. 2006. "P25alpha/Tubulin Polymerization Promoting Protein Expression by Myelinating Oligodendrocytes of the Developing Rat Brain." *Journal of Neurochemistry* 99: 333–342.

Song, Y. J., D. M. Lundvig, Y. Huang, et al. 2007. "p25alpha Relocalizes in Oligodendroglia From Myelin to Cytoplasmic Inclusions in Multiple System Atrophy." *American Journal of Pathology* 171: 1291–1303.

Spillantini, M. G., M. L. Schmidt, V. M. Lee, J. Q. Trojanowski, R. Jakes, and M. Goedert. 1997. "Alpha-Synuclein in Lewy Bodies." *Nature* 388: 839–840.

Štifanić, M., R. Batel, and W. E. G. Müller. 2011. "Tubulin Polymerization Promoting Protein (TPPP) Ortholog From Suberites Domuncula and Comparative Analysis of TPPP/p25 Gene Family." *Biologia* 66: 111–120.

Suk, T. R., and M. W. C. Rousseaux. 2020. "The Role of TDP-43 Mislocalization in Amyotrophic Lateral Sclerosis." *Molecular Neurodegeneration* 15: 45.

Szabo, A., J. Olah, S. Szunyogh, et al. 2017. "Modulation of Microtubule Acetylation by the Interplay of TPPP/p25, SIRT2 and New Anticancer Agents With Anti-SIRT2 Potency." *Scientific Reports* 7: 17070.

Szunyogh, S., J. Olah, T. Szenasi, A. Szabo, and J. Ovadi. 2015. "Targeting the Interface of the Pathological Complex of Alpha-Synuclein and TPPP/p25." *Biochimica et Biophysica Acta* 1852: 2653–2661.

Takahashi, M., K. Tomizawa, K. Ishiguro, et al. 1991. "A Novel Brain-Specific 25 kDa Protein (p25) is Phosphorylated by a Ser/Thr-Pro Kinase (TPK II) From Tau Protein Kinase Fractions." *FEBS Letters* 289: 37–43.

Takahashi, M., K. Tomizawa, S. C. Fujita, K. Sato, T. Uchida, and K. Imahori. 1993. "A Brain-Specific Protein p25 Is Localized and Associated With Oligodendrocytes, Neuropil, and Fiber-Like Structures of the CA3 Hippocampal Region in the Rat Brain." *Journal of Neurochemistry* 60: 228–235.

Tirian, L., E. Hlavanda, J. Olah, et al. 2003. "TPPP/p25 Promotes Tubulin Assemblies and Blocks Mitotic Spindle Formation." *Proceedings of the National Academy of Sciences of the United States of America* 100: 13976–13981.

Tokesi, N., A. Lehotzky, I. Horvath, et al. 2010. "TPPP/p25 Promotes Tubulin Acetylation by Inhibiting Histone Deacetylase 6." *Journal of Biological Chemistry* 285: 17896–17906.

Tokesi, N., J. Olah, E. Hlavanda, et al. 2014. "Identification of Motives Mediating Alternative Functions of the Neomorphic Moonlighting TPPP/p25." *Biochimica et Biophysica Acta* 1842: 547–557.

Tripon, R. G., J. Olah, T. Nasir, et al. 2018. "Localization of the Zinc Binding Tubulin Polymerization Promoting Protein in the Mice and Human Eye." *Journal of Trace Elements in Medicine and Biology* 49: 222–230.

Vargas, E. J. M., A. J. Matamoros, J. Qiu, et al. 2020. "The Microtubule Regulator Ringer Functions Downstream From the RNA Repair/Splicing Pathway to Promote Axon Regeneration." *Genes & Development* 34: 194–208.

Vincze, O., J. Olah, D. Zadori, P. Klivenyi, L. Vecsei, and J. Ovadi. 2011. "A New Myelin Protein, TPPP/p25, Reduced in Demyelinated Lesions Is Enriched in Cerebrospinal Fluid of Multiple Sclerosis." *Biochemical and Biophysical Research Communications* 409: 137–141.

Vincze, O., N. Tokesi, J. Olah, et al. 2006. "Tubulin Polymerization Promoting Proteins (TPPPs): Members of a New Family With Distinct Structures and Functions." *Biochemistry* 45: 13818–13826.

Wakabayashi, K., Y. Miki, K. Tanji, and F. Mori. 2024. "Neuropathology of Multiple System Atrophy, a Glioneuronal Degenerative Disease." *Cerebellum* 23: 2–12.

Wang, W., S. Tian, H. Nie, et al. 2021. "CFAP65 Is Required in the Acrosome Biogenesis and Mitochondrial Sheath Assembly During Spermiogenesis." *Human Molecular Genetics* 30: 2240–2254.

Wenning, G. K., N. Stefanova, K. A. Jellinger, W. Poewe, and M. G. Schlossmacher. 2008. "Multiple System Atrophy: A Primary Oligodendrogliopathy." *Annals of Neurology* 64: 239–246.

Xie, J., S. Chen, J. C. Bopassa, and S. Banerjee. 2021. "*Drosophila* Tubulin Polymerization Promoting Protein Mutants Reveal Pathological Correlates Relevant to Human Parkinson's Disease." *Scientific Reports* 11: 13614.

Yang, Y., Y. Shi, M. Schweighauser, et al. 2022. "Structures of Alpha-Synuclein Filaments From Human Brains With Lewy Pathology." *Nature* 610: 791–795.

Youn, Y. H., and Y. G. Han. 2018. "Primary Cilia in Brain Development and Diseases." *American Journal of Pathology* 188: 11–22.

Zhang, K., C. J. Donnelly, A. R. Haeusler, et al. 2015. "The C9orf72 Repeat Expansion Disrupts Nucleocytoplasmic Transport." *Nature* 525: 56–61.

Zhou, R. M., Y. Y. Jing, Y. Guo, et al. 2011. "Molecular Interaction of TPPP With PrP Antagonized the CytoPrP-Induced Disruption of Microtubule Structures and Cytotoxicity." *PLoS One* 6: e23079.

Zhu, F., P. Yan, J. Zhang, et al. 2019. "Deficiency of TPPP2, a Factor Linked to Oligoasthenozoospermia, Causes Subfertility in Male Mice." *Journal of Cellular and Molecular Medicine* 23: 2583–2594.

Zotter, A., J. Olah, E. Hlavanda, et al. 2011. "Zn(2)+−induced Rearrangement of the Disordered TPPP/p25 Affects Its Microtubule Assembly and GTPase Activity." *Biochemistry* 50: 9568–9578.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.