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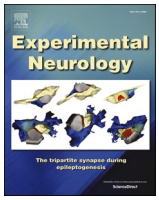

Review article

Cross-talk between α-synuclein and the microtubule cytoskeleton in neurodegeneration

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ABSTRACT

Looking at the puzzle that depicts the molecular determinants in neurodegeneration, many pieces are lacking and multiple interconnections among key proteins and intracellular pathways still remain unclear. Here we focus on the concerted action of α-synuclein and the microtubule cytoskeleton, whose interplay, indeed, is emerging but remains largely unexplored in both its physiology and pathology. α-Synuclein is a key protein involved in neurodegeneration, underlying those diseases termed synucleinopathies. Its propensity to interact with other proteins and structures renders the identification of neuronal death trigger extremely difficult. Conversely, the unbalance of microtubule cytoskeleton in terms of structure, dynamics and function is emerging as a point of convergence in neurodegeneration. Interestingly, α-synuclein and microtubules have been shown to interact and mediate cross-talks with other intracellular structures. This is supported by an increasing amount of evidence ranging from their direct interaction to the engagement of in-common partners and culminating with their respective impact on microtubule-dependent neuronal functions. Last, but not least, it is becoming even more clear that α-synuclein and tubulin work synergically towards pathological aggregation, ultimately resulting in neurodegeneration. In this respect, we supply a novel perspective towards the understanding of α-synuclein biology and, most importantly, of the link between α-synuclein with microtubule cytoskeleton and its impact for neurodegeneration and future development of novel therapeutic strategies.

1. Introduction

Biological mechanisms of neurodegeneration are extremely complex, as discussed for Parkinson's disease (Panicker et al., 2021). However, molecular determinants responsible for neuronal damage first and foremost warrant to be defined being the *conditio sine qua non* for the design of an efficient strategy for both neuroprotection and potential therapies.

In the context of neuropathologies, α-synuclein is, to some extent, a "master regulator" for neurodegeneration. α-Synuclein's established involvement in numerous neurodegenerative disorders (Parkinson's disease, dementia with Lewy bodies, multiple system atrophy and pure autonomic failure), has led to the classification of these as synucleinopathies. However, beyond synucleinopathies, emerging evidence links α-synuclein to Alzheimer's disease and tauopathies (Twohig and Nielsen, 2019). Further, recent investigations have broadened the involvement of α-synuclein to neurodevelopmental disorders, including autism spectrum disorders (Morato Torres et al., 2020), and epileptic seizure (Hussein et al., 2019). These recent investigations suggest its broad association with numerous pathways which underpin the overall health of the nervous system. This is thought to be driven by its physico-chemical characteristics. α-Synuclein is a 14 kDa soluble protein which exists as intrinsically unfolded, and thus highly predisposed towards the adoption of multiple conformations. Such tendencies catalyse its interaction with several partners (Lassen et al., 2016). As briefly mentioned, there are many potential partners and pathways that could trigger α-synuclein-associated neuronal damage. Here we focus on its interplay with microtubules.

Unbalance of microtubules is becoming a point of convergence in neurodegeneration (Matamoros and Baas, 2016). Neuron's morphological integrity and physiological function strictly rely on a healthy microtubule system (Kapitein and Hoogenraad, 2015). More specifically, homeostasis of neuronal microtubules depends on fine regulation of their dynamics (Rolls et al., 2021) and post-translational

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modifications (Moutin et al., 2021). This, in turn, contributes to sustain microtubule-dependent functions in somato-dendritic, axonal, and synaptic compartments with axonal transport role (Guedes-Dias and Holzbaur, 2019) and synaptic activity (Qu et al., 2019). In light of such functions, it is not surprising that microtubular defects have been linked to a wide range of neurodegenerative diseases: Charcot-Marie-Tooth disease (d'Ydewalle et al., 2011), Hereditary Spastic Paraplegia (Roll-Mecak and Vale, 2008), Alzheimer's disease (Brandt and Bakota, 2017), and Parkinson's diseases (Cappelletti et al., 2021; Cartelli and Cappelletti, 2017; Pellegrini et al., 2017).

To date, understanding cross-talks between α-synuclein and microtubule in the context of neurodegeneration can contribute towards the construction of a novel model capable of elucidating the physico-chemical mechanisms which lead to irreversible neuronal damage.

2. α-Synuclein: a "driving force" in neurodegeneration?

Two seminal papers published in 1997 linked α-synuclein to neurodegeneration. The first fundamental study evidences a mutation in *SNCA*, the gene coding for α-synuclein, as a driver of an autosomal early-onset form of Parkinson's disease (Polymeropoulos et al., 1997) giving rise to the so-called "genetic era" for Parkinson's disease. The second paper demonstrates the localization of α-synuclein within Lewy bodies, suggesting that these structures are the major repository of such protein aggregations that are the pathological hallmarks of Parkinson's disease (Spillantini et al., 1997). As a result of these two key findings, over the last 20 years, α-synuclein has been the focus of numerous studies dissecting synucleinopathies (Goedert et al., 2017). α-Synuclein is a "natively unfolded" protein, since a peculiar characteristic is its intrinsic disordered structure (Uversky, 2002). In physiological conditions, it has been proposed that α-synuclein is mainly present as unfolded monomers in neurons (Burré et al., 2013). α-Synuclein protein is characterized by three main domains: the N-terminal region (residues 1-60), the non-amyloid-β component (NAC; residues 61-96), and the C-terminal domain (residues 97-140). While the N-terminal and NAC domains contain seven imperfect repeats of the consensus sequence KTKEGV, the C-terminal domain lacks them and is enriched in negatively charged amino acids and proline residues, which are known to disrupt secondary protein structure (George et al., 1995; Ulmer et al., 2005). Moreover, α-synuclein shows 18 glycine residues, distributed along the entire sequence, that, with a hydrogen side chain, exhibit minimal conformational restrictions on protein folding. It is thought that all these features increase the flexibility of the protein and contribute to its intrinsically disordered nature (Holec et al., 2022). In detail, structural studies indicate that the N-terminal region folds into two α-helices connected by a linker sequence when α-synuclein bound to acidic lipids, while the C-terminal domain remains disordered. On the contrary, in pathological conditions (*i.e.* in synucleinopathies) this natively unfolded protein is able to acquire β-sheet-rich structures, in which the central region (composed by the last residues of the N-terminal domain and the NAC domain) became structured while the peripheral regions remain unstructured. The mechanisms of synucleinopathies involve the aggregation of α-synuclein into several forms (oligomers and fibrils) in both neurons and glial cells. However, stemming from the findings of Spillantini and co-workers (Spillantini et al., 1997), α-synuclein fibrils have been found to be the main components of Lewy bodies in neurons of Parkinson's disease and dementia with Lewy bodies patients. Additionally, α-synuclein fibrils have also been identified in Papp-Lantos bodies localized in glial cells of multiple system atrophy patients (Dickson, 2012), and were found to be predominantly phosphorylated in pathological lesions (Fujiwara et al., 2002). Interestingly, phosphorylated α-synuclein has been also evidenced in nerve fibers of the autonomic nervous system in skin biopsies obtained from synucleinopathies affected patients and also in pure autonomic failure (Donadio et al., 2013). Beyond fibrils, increasing attention has been paid on α-synuclein oligomers, regarded as the most toxic species (Bengoa-Vergniory et al., 2017; Wong and Krainc, 2017). Oligomers of α-synuclein have been identified with the aid of proximity ligation assays in neurons of both brain (Roberts et al., 2015) and skin biopsy (Mazzetti et al., 2020a, 2020b) obtained from patients affected with Parkinson's disease. Interestingly, in multiple system atrophy brains, α-synuclein oligomers were present in glial cells, but were also abundant in neurons, demystifying atrophy of numerous nuclei of brain stem neurons and Purkinje cells within cerebellum (Sekiya et al., 2019).

The role and extensive implications of α-synuclein oligomers and fibrils on neuronal and glial activities is emerging. Studies using multiple models and approaches revealed that α-synuclein aggregates exert a variety of deleterious effects, including mitochondrial (Archer, 2013; Devi et al., 2008; Schapira, 2007) and Calcium homeostasis alterations (Mosharov et al., 2009; Surmeier et al., 2017), oxidative stress (Anderson and Kedersha, 2002; Salemi et al., 2021), as well as impairment of both the ubiquitin proteasome system and the autophagy lysosomal pathway (Mazzetti et al., 2020a; Olanow et al., 2004; Pan et al., 2008). Collectively, these alterations lead to overall inflammation that involves glial cells (Hirsch and Hunot, 2009; Mazzetti et al., 2022; Ransohoff, 2016).

It is important to mention that α-Synuclein aggregation occurs alongside abnormal deposits of other proteins (Visanji et al., 2017) that are classically indicated as the main hallmark of other proteinopathies, including: tau (Holmes et al., 2014; Yan et al., 2018), transactive response DNA binding protein 43 kDa (TDP-43; (Koga et al., 2018)), amyloid-β (Aβ; (Swirski et al., 2014)), or prions (Haïk et al., 2002). This suggests that α-synuclein aggregation could be crucial not only in synucleinopathies but also in pathologic comorbidities of other neurodegenerative diseases, including tauopathies, Alzheimer's disease, amyotrophic lateral sclerosis and prion disease (Visanji et al., 2019). However, two important questions still remain largely unanswered: *i)* whether α-synuclein could be defined as the "main driver" in neurodegeneration; and *ii)* how it could play this role. We believe that the answers will come from understanding the physiological partners and functions of α-synuclein that, indeed, are focal points within the scientific community. In the context of α-synuclein biology, we attempt to elucidate whether its loss of function could be linked to aggregation and, consequently, be the trigger or the hallmark of neurodegeneration. Here we focus on emerging partners of α-synuclein, tubulin and microtubules, with a different perspective towards neurodegenerative pathologies.

3. α-Synuclein and tubulin: partners in health

The first evidence of a link between α-synuclein and microtubule cytoskeleton dates to the beginning of this millennium, when α-synuclein was found to co-immunoprecipitate with α- and β-tubulin in zebra finch and murine forebrains (Payton et al., 2001). During the last twenty years, this issue was not intensively addressed, but significant amounts of experimental evidence have been produced to strengthen this link and understand its meaning, thus adding little but important pieces to the puzzle (Calogero et al., 2019). Before looking in detail at the data that support the cross-talk between α-synuclein and tubulin/microtubules in physiological condition, we cite the key features regarding the microtubule organization and homeostasis in neurons. Polarity, stability, and interaction with specific microtubule-associated proteins are essential to make microtubules unique for achieving the extraordinary morphological, mechanical, and functional complexity of the neuron. The axonal and somato-dendritic compartments display a uniform and mixed-oriented microtubule array, respectively, which establishes their different polarity and determines the binding of plus and minus end-directed molecular motor and, consequently, anterograde and retrograde transport of proteins, mRNA, and organelles, as well as their sorting (Aiken and Holzbaur, 2021). In addition, the stability of microtubules marks out neuronal compartments as stabilized and dynamic microtubules are differentially present in axons, dendrites, and synapses where they both shape microtubule arrays and control microtubule-

dependent functions (Baas et al., 2016; Waites et al., 2021). Pivotal role for regulation of microtubule polarity, stability, and their dependent intraneuronal processes are both post-translational modifications of tubulin and the activity of a plethora of microtubule-associated proteins (Janke and Magiera, 2020). In this context, understanding which is the role of α-synuclein as a partner of tubulin and how it impacts on neuronal microtubules is challenging.

3.1. Evidence for the direct interaction of α-synuclein with tubulin/microtubules

The concept of potentially direct interaction between α-synuclein and tubulin/microtubules in neurons was firstly suggested by Payton et al. (2001), and then developed thanks to the work of other teams. α-Synuclein and tubulins co-immunoprecipitate from both human brain and rat brain extracts and, furthermore, they co-purify with soluble tubulin from rat brain following a restrictive purification procedure (Alim et al., 2004; Alim et al., 2002). The association between α-synuclein and different isoforms of tubulins was further supported by quantitative proteomics in rat MES cells (Zhou et al., 2004). Further experimental evidence coming from mouse and rat nervous system (Amadeo et al., 2021; Toba et al., 2017) and in *post-mortem* human brain (Amadeo et al., 2021). In detail, α-synuclein co-localizes and is in close proximity to α-tubulin in murine and human brain as revealed by confocal and electron microscopy analysis (Amadeo et al., 2021). Next, focusing on microtubules, α-synuclein binding to *in vitro* assembled microtubules has been revealed (Toba et al., 2017). However, although the above-described evidence strongly suggests the direct interaction of α-synuclein with tubulin, further data are required to consolidate the hypothesis that α-synuclein is a true microtubule associated protein, namely a protein able to bind tubulin or microtubules and regulate their behaviour.

Certainly, at least the direct binding between α-synuclein and β-III tubulin has been demonstrated *in vitro* using two human proteins purified from *E. coli*, thus suggesting that indirect binding, from other proteins, could be excluded (Suzuki et al., 2014). However, it is important to consider that proteins purified from *E. coli* are completely undressed from all those post translational modifications which are known to exert a fundamental role in the regulation of tubulin functions and interactions (Janke and Magiera, 2020). For solving the open question and get an insight into the molecular basis of the potential direct interaction between α-synuclein and tubulin, it is crucial to further investigate such interaction with the aid of advanced analytical approaches and tubulin purified by mammalian cells or organs. With this knowledge, the formation of a complex between α-synuclein and soluble tubulin has been demonstrated by native mass spectrometer/nano-electrospray ionization and NMR diffusion measurements approaches (Cartelli et al., 2016), and by surface plasmon resonance (Toba et al., 2017). On the other hand, crystal structures of the α-synuclein-tubulin complex have yet to be acquired due to the intrinsically unstructured nature of α-synuclein that makes the formation of a stable complex highly improbable. From the available literature, different groups propose distinct regions of α-synuclein to be involved in its interaction with tubulin/microtubules, including mainly N-terminal region (Cartelli et al., 2016), NAC region (Zhou et al., 2010), or C-terminal region (Alim et al., 2004). Vast differences in proposed regions of interest suggest that the entire α-synuclein sequence could be involved in such interactions. Further studies, however, warrant to investigate further the residues which could be responsible for direct interaction between α-synuclein and tubulin/microtubules.

3.2. Evidence for the indirect interaction of α-synuclein with tubulin

Looking at the other side of the coin, the interplay between α-synuclein and tubulin/microtubules could be indirect and likely mediated by common partners (Carnwath et al., 2018). Indeed, a plethora of protein partners of α-synuclein were found in cellular compartments, from presynapse and mitochondria to endoplasmic reticulum, and are involved in both metabolic and catabolic processes (Burré et al., 2018; Lassen et al., 2016). Interestingly, some of them have the potential to play a role in the proposed interaction between α-synuclein and tubulin/microtubules. The first candidate is tau protein that has been shown to be an α-synuclein ligand in human brain cytosol by affinity chromatography. Their interacting domain was evidenced, using protein fragmentation and recombinant peptides, in C-terminus of both α-synuclein and tau, the same region where tau includes the repeated microtubule binding domains (Jensen et al., 1999). Interestingly, α-synuclein promotes tau phosphorylation, and thus can indirectly affecting microtubules (Jensen et al., 1999). In fact, tau is a well-known microtubule-associated protein (MAP), involved in regulating microtubule dynamic instability (Wang and Mandelkow, 2016) and in enabling axonal microtubules to have long labile domains (Baas and Qiang, 2019). It is now established that tau plays crucial synergistic effects with α-synuclein in neurons and mediates cross-talk essential in maintaining neuronal homeostasis, including the regulation of microtubules' stability and polymerization (Vacchi et al., 2020). Notably, other MAPs have been identified as α-synuclein interactors by a proteomic screen, including: MAP1B, MAP2 and MAP6 (Betzer et al., 2015), although MAP1B is reported to preferentially bind α-synuclein fibrils (Jensen et al., 2000). A second group of putative in-common partners of α-synuclein and microtubules are molecular motors. Indeed, α-synuclein needs to be rapidly transported along microtubules to reach synapses. Studies on α-synuclein in rat cortical primary neurons using live cell imaging and co-immunoprecipitation techniques demonstrated its link with both kinesin 1 and dynein (Utton et al., 2005). Toba and co-workers also demonstrated that α-synuclein's interaction with βIII-tubulin occurs in rat nerve, where, together with dynein, they form a complex that allow the transport of short microtubules (Toba et al., 2017). On the contrary, in human brain neurons, α-synuclein transport by dynein has been suggested to occur *via* histone deacetylase 6 (HDAC6). These results were suggested as proximity ligation assay failed to reveal the direct association of α-synuclein with dynein (Mazzetti et al., 2020a). Finally, additional candidates proposed as common partners between α-synuclein and tubulin/microtubules could belong to the family of prolyl oligopeptidase enzymes, such as prolyl oligopeptidase or prolyl endopeptidase PREP/PEP (Gerard et al., 2010; Kilpeläinen et al., 2020). PREP directly interacts with α-synuclein *in vitro* and in cells, and thus a protein-protein interaction increases α-synuclein dimerization independently of PREP proteolytic activity (Savolainen et al., 2015). On the other hand, PREP is involved in regulating microtubule dynamics (Chambraud et al., 2022; Schulz et al., 2005). PREP specifically was implicated in the cellular transport and protein secretion independent of its peptidase function (Schulz et al., 2005). This data suggests that PREP could bridge α-synuclein and microtubule network.

To conclude, direct and indirect interaction of α-synuclein with tubulin/microtubes are likely and, in principle, are not mutually exclusive. Thus, a more complete theory should consider that both might contribute to neuronal health, for which α-synuclein could exert part of its functions thanks to its direct binding to tubulin/microtubules, while additional functions can occur thanks to its interaction with other tubulin/microtubule binding proteins.

4. α-Synuclein and tubulin interplay: From health to neuronal dysfunction

Trying to dissect whether and how α-synuclein interplay with tubulin/microtubules contributes to neuronal dysfunction and, ultimately, to neurodegeneration, two major issues must be assessed: the maintenance/loss of their interaction, both direct and mediated by in-common partners, and the impact on microtubule system behaviour and related function. The emerging data from *in vitro* and in mouse disease models that support this analysis are summarized in Table 1.

Table 1
Evidence for the cross-talk between α-synuclein and tubulin/microtubules in synucleinopathies.

Disease	Experimental model	Results	References
Parkinson's disease	*In vitro* assays using purified α-synucleins (wild-type and mutated variants linked to Parkinson's disease) and purified tubulin	• Mutated forms display defective interaction with tubulin[1] • Mutated forms do not undergo tubulin-induced folding[2] • Mutated forms interfere with tubulin assembly[3] • Tubulin accelerates α-synuclein aggregation into fibrils [4]	[1]Cartelli et al., 2016; Toba et al., 2017 [2]Cartelli et al., 2016 [3]Alim et al., 2004; Zhou et al., 2010; Toba et al., 2017 [4]Alim et al., 2002; Kim et al., 2008; Nakayama et al., 2012
	Mouse primary neurons overexpressing α-synuclein	• Disruption of the microtubule network and impairment of microtubule-dependent trafficking[1]	[1]Lee et al., 2006
	MPP^+-treated murine microglia cells	• The activation of inflammasome by α-synuclein occurs trough microtubule-driven mechanisms[1]	[1]Xueping et al., 2022
	Brain slices from wild type mice	• Mutated α-synucleins (A30P and A53T) lose the ability to regulate vesicle endocytosis *via* microtubule over-assembly in presynaptic terminals[1]	[1]Eguchi et al., 2017
	MPTP-treated and α-synuclein overexpressing mice	• α-Synuclein induces hyperphosphorylation of the microtubule binding protein tau[1]	[1]Duka et al., 2009
	Transgenic mice overexpressing A53T α-synuclein	• The synergic action of α-synuclein and microtubules promotes the mutual polymerization into fibrils[1]	[1]Giasson et al., 2003
	Human cybrid cells	• Loss of microtubules mass influences α-synuclein aggregation[1]	[1]Esteves, 2010
	Human iPSC-derived dopaminergic neurons of patients	• Oligomeric α-synuclein preferentially binds tubulin isoforms, as revealed by unbiased mass spectrometry [1]	[1]Seebauer et al., 2022
	Post-mortem human brain	• Presence of tubulin in α-synuclein positive Lewy bodies[1] • The main tubulin deacetylase (HDAC6) co-localizes with α-synuclein in Lewy bodies and contributes to Lewy body morphogenesis [2]	[1]Galloway et al., 1992; Alim et al., 2002; Moors et al., 2021 [2]Mazzetti et al., 2020a; Moors et al., 2021
Multiple system atrophy	Primary neurons from transgenic mice overexpressing human α-synuclein in oligodendrocytes	• α-Synuclein binds βIII-tubulin and triggers aggregation. Drug-induced depolymerization of microtubules suppresses aggregation[1] • Accumulation of α-synuclein is impaired by using a peptide of tubulin that specifically binds α-synuclein [2]	[1]Nakayama et al., 2009, Nakayama et al., 2012; Ito et al., 2012 [2]Suzuki et al., 2014
	Brain of transgenic mice overexpressing human α-synuclein in oligodendrocytes; *Post-mortem* human brain	• Co-localization of βIII-tubulin with α-synuclein [1]	[1]Suzuki et al., 2014
Dementia with Lewy bodies	*Post-mortem* human brain	• Co-localization tubulin/α-synuclein [1]	[1]Iseki et al., 2000

Firstly, we hypothesise that alteration of the interaction between α-synuclein and tubulin could be directly linked to a pathogenic process. *In vitro* data obtained through different approaches indicate that mutations related to Parkinson's disease compromise the ability of α-synuclein to interact with tubulin/microtubules. In fact, A30P and E46K mutations completely suppressed the binding affinity of the protein to tubulin, as well as affecting microtubule assembly (Toba et al., 2017). Also, A30P, A46K, and A53T α-synuclein variants exhibit changes in folding as they do not undergo tubulin-induced folding (Cartelli et al., 2016). Furthermore, we pointed out that all the suggested putative microtubule binding domains on α-synuclein overlap with regions that are involved in its self-aggregation (Calogero et al., 2019), being located as previously mentioned, in the central region able to acquire β-sheet structures (Cartelli et al., 2016; Zhou et al., 2010), or located in the C-terminal region (Alim et al., 2004). Interestingly, C-terminal region seems to interact with the N-terminal region of α-synuclein in order to protect the aggregation-prone NAC domain, relevant towards the formation of compact aggregation-resistant monomeric structures (Bertoncini et al., 2005; Dedmon et al., 2005). On the basis that α-synuclein binding to microtubules leads to acquisition of α-helices structures in physiological condition (Cartelli et al., 2016) and considering the high structural flexibility of α-synuclein and its predisposition to aggregate when the NAC domain is available, its proper interaction with its physiological partners, and therefore also with tubulins, can be required to avoid α-synuclein fold rearrangements that lead to self-aggregation (Fig. 1).

A second branch of evidence stems from studies on Tubulin Polymerization Promoting Protein (TPPP/p25), a brain-specific protein involved in the regulation of dynamics and stability of the microtubule system and capable of inducing microtubule bundling (Lehotzky et al., 2004). Pathologically, TPPP/p25α binds α-synuclein at its C-terminus region and forms α-synuclein oligomers/aggregates in neurons and oligodendrocytes in Parkinson's disease and multiple system atrophy, respectively (Oláh et al., 2020). The physiological role, if any, resulting from the interaction of TPPP/p25α with α-synuclein is still unknown as this complex has been detected exclusively at pathological conditions where defects in regulating microtubules are also observed. Thus, the role played by TPPP/p25α could be attributable to it being a common partner for both α-synuclein and microtubules.

A third branch of evidence links the interplay between α-synuclein and tubulin with neurodegeneration. This has been suggested following studies on neuronal functions that rely on healthy microtubule system. The physiological outcome of the α-synuclein interplay with tubulin/microtubules includes the regulation of microtubule behaviour in terms of assembly kinetics and dynamics, as well as axonal transport.

4.1. Impact on microtubule dynamics

Right from the beginning, it has been demonstrated that α-synuclein influences tubulin assembly kinetics both promoting (Alim et al., 2004; Alim et al., 2002; Toba et al., 2017) and inhibiting microtubule formation (Zhou et al., 2010). Subsequently, a more detailed view of such behaviour suggests that α-synuclein *i)* acts as a dynamase, due to its ability to regulate both microtubule nucleation and catastrophe in cell-

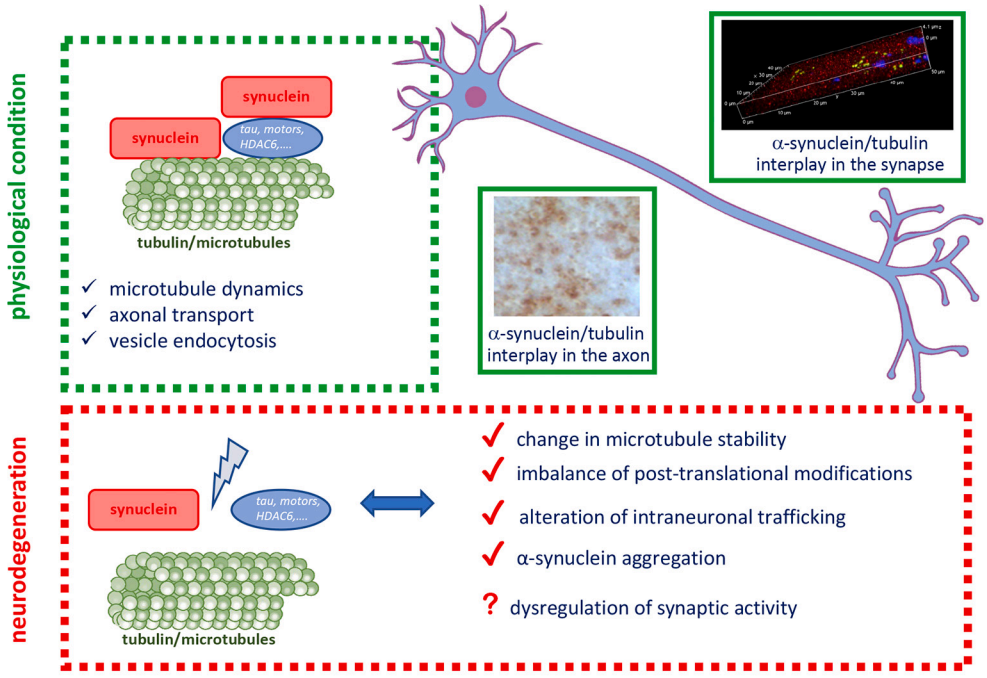

Fig. 1. Proposed cross-talk of α-synuclein and tubulin/microtubules in neurodegeneration.
In physiological condition, α-synuclein interacts with tubulin in both axonal and synaptic compartments. Confocal microscopy images show the close association between α-synuclein and tubulin, as visualized using proximity ligation assay, in axons of control human brain (brown signal) and in synaptic terminals of mouse brain (green signal: α-synuclein/tubulin association; red signal: synaptophysin) (see Amadeo et al., 2021 for details). Evidence exists that such an interplay could be direct o mediated by in-common partners of α-synuclein and tubulin/microtubules (including protein tau, molecular motors, and the main α-tubulin deacetylase HDAC6). The impact of this cross-talk is reported in the regulation of microtubule dynamics, microtubule-dependent processes as axonal transport, and vesicle recycling. Additional neuronal processes modulated by this interplay remain to be disclosed.
In neurodegeneration, emerging data suggest that the interplay between α-synuclein and tubulin/microtubules is weakened or disrupted. This has the potential to dramatically affect neuronal function, being the cause or the consequence of changes in microtubule stability, imbalance of post-translationally modified tubulins and microtubules, impairment of intraneuronal transport, and α-synuclein aggregation. It has left a question over whether and how α-synuclein interplay with tubulin/microtubules plays a role in synaptic dysfunction underlying neurodegeneration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

free systems; *ii)* increases microtubule growth rate in cells; and *iii)* colocalizes with tyrosinated α-tubulin, which is associated with the most dynamic microtubules, in human neurons derived from embryonic stem cells expressing endogenous levels of α-synuclein itself (Cartelli et al., 2016). Moving to the context of synucleinopathies, the interference of α-synuclein with tubulin assembly kinetics and dynamics has the potential to be a key player in the pathogenesis. Microtubule assembly is reduced by A30P, A53T or E46K variants when compared to wild-type protein in cell free systems (Alim et al., 2004; Toba et al., 2017), and in cells (Cartelli and Cappelletti, 2017).

4.2. Effect on axonal transport

α-Synuclein overexpression in primary neurons correlates with disruption of the microtubule network, impairment of microtubule dependent trafficking and neurite degeneration (Lee et al., 2006). To note, α-synuclein has been demonstrated to play a role also in tau aggregation leading to microtubule instability and axonal transport impairment (Giasson et al., 2003; Jensen et al., 1999; Torres-Garcia et al., 2022). α-Synuclein induces tau phosphorylation *via* protein kinase A on serine residues 262 and 356 (Jensen et al., 1999), and also *via* glycogen synthase kinase 3 beta, as demonstrated in the MPTP-treated mouse model of Parkinson's disease (Duka et al., 2009).

Beyond models of Parkinson's disease, some interesting data have been gathered from a transgenic mouse model of multiple system atrophy in which human wild-type α-synuclein is selectively expressed in oligodendrocytes and has been shown to result in neuronal degeneration. In primary cells obtained from this model, it has been demonstrated that binding of α-synuclein to β-III tubulin caused the formation of insoluble complexes. Both the formation and accumulation of these α-synuclein insoluble aggregates was shown to be suppressed by microtubule depolymerization induced by nocodazole (Nakayama et al., 2009, 2012). Interestingly, treatment with nocodazole attenuates pathological processes in this mouse model (Ito et al., 2012), which suggests that the binding of α-synuclein to β-III tubulin is a key process in the development of neuronal degeneration in multiple system atrophy.

4.3. Impact on synapses

The important role and the main localization of α-synuclein inside presynaptic terminals rise the questions of whether the interplay between α-synuclein and microtubules occurs inside this compartment, and which could be its physiological/pathological impact. First and foremost, it is important to state that the presence of microtubules inside synapses is a long-lasting controversial issue (Dent, 2020). The difficulties associated with microtubule visualization within the synaptic compartment is a direct consequence of their intrinsic instability and scarcity. Nonetheless, their presence was highlighted for the first time in presynapses of central nervous system, specifically in the varicosities of the autonomic nervous system and in rat brain synaptosomes only by using specific fixatives enriched in EGTA. The addition of these fixatives enabled prevention of their disassembly by Calcium influx (Gordon-Weeks et al., 1982). More recently, microtubule visualization was evinced by means of live cell imaging studies in *Drosophila* which showed microtubules inside synaptic boutons (Bodaleo and Gonzalez-Billault, 2016). It has been suggested that the role of α-synuclein in maintaining recycling pool vesicles homeostasis relies on its ability of modulating intersynaptic vesicular dynamics and is related also to its interaction with microtubules (Scott and Roy, 2012). More recently, in mammalian neurons, microtubule's functional role was demonstrated in the excitatory *en passant* varicosities release (Qu et al., 2019). Looking at this evidence and using multiple approaches, from proximity ligation assay to electron microscopy techniques, the interplay between α-synuclein and tubulin was confirmed inside presynaptic terminals in both mouse and human brain (Amadeo et al., 2021). This discovery suggested that the presynapse could be the compartment in which α-synuclein

modulates synaptic release and activity also *via* regulation of microtubules, due to its dynamase activity (Cartelli et al., 2016). Interestingly, a study on the calyces of Held presynaptic terminals in brain slices from mature rats revealed that wild-type α-synuclein, but not A53T and A30P variants, primarily inhibits vesicle endocytosis *via* microtubule overassembly, thereby impairing neurotransmission (Eguchi et al., 2017).

All together these experimental data support our hypothesised association between α-synuclein and microtubules system in the different neuronal compartments, an association which could play a role in triggering neuronal dysfunction.

5. α-Synuclein and tubulin work synergically in pathological aggregation

Beyond the potential to trigger neurodegeneration *via* the regulation of tubulin assembly and dynamics and the impairment of microtubule-based functions, α-synuclein could work side by side with tubulin in the formation of protein aggregates that mark synucleinopathies.

Historically, the first attempt to define the molecular composition of Lewy bodies revealed that they are composed of filamentous inclusions and contain cytoskeletal proteins, including tubulin, MAPs, and neurofilaments (Galloway et al., 1992). The same authors that discovered the link between α-synuclein and tubulin suggested that tubulin could be involved in Parkinson's disease pathology. This hypothesis was supported by their demonstration that α-tubulin colocalizes with α-synuclein within brains of patients, at the level of both Lewy bodies and Lewy neurites (Alim et al., 2002).

Regarding the other synucleinopathies, α-Synuclein co-localizes with β-III tubulin in the brain tissues of patients with multiple system atrophy (Suzuki et al., 2014), while Lewy bodies positive for tubulin were also found in DLB brains (Iseki et al., 2000). Notably, by employing a proteomic screen, three tubulin chains have been identified as preferential interactors of oligomeric α-synuclein, namely α-4a, β-3, and α β-4 tubulins (Betzer et al., 2015). In line with this evidence, α-synuclein aggregates isolated from cells overexpressing wild-type α-synuclein and human iPSC-derived dopaminergic neurons of Parkinson's disease patients were analysed by means of mass spectrometry. These studies revealed that the preferential binding of such aggregated conformers occurs towards a number of tubulin isoforms (Seebauer et al., 2022). Even if the presence of tubulins in insoluble aggregates of α-synuclein is evidenced, it does not directly imply that they play a pathogenic role. In fact, few studies have been published which demonstrate that tubulin could affect α-synuclein aggregation. Indeed, *in vitro* experiments indicate that α-tubulin can induce and accelerate α-synuclein aggregation into fibrils (Alim et al., 2002; Kim et al., 2008). This is true also for β-III tubulin, which is able to increase α-synuclein aggregation in a concentration-dependent manner (Nakayama et al., 2012). Moving to cellular models of Parkinson's disease, evidence exists suggesting that the loss of microtubule mass influences α-synuclein aggregation (Esteves, 2010), and that the regulation of tubulin acetylation using both pharmacological and genetic inhibition of the deacetylase SIRT2 reduces the formation of α-synuclein inclusions and alleviates neurotoxicity (Outeiro et al., 2007). Also, many studies support the proposed role of HDAC6, as the main deacetylase that specifically targets tubulin as a non-histone substrate, on α-synuclein aggregation. Nonetheless, the behaviour observed with HDAC6 could be attributable to the acetylation of tubulin and/or protein clearance mechanisms (Lemos and Stefanova, 2020). Importantly, the use of a short peptide fragment of β-III tubulin, which specifically binds α-synuclein, suppresses α-synuclein accumulation in primary cultured neurons from multiple system atrophy mice model (Suzuki et al., 2014).

Albeit understanding the underlying mechanisms which dictate the build-up of protein aggregates remains a challenge, the strategy proposed to investigate the morphogenetic pathways triggering monomeric α-synuclein towards mature Lewy bodies, is promising. Moors and colleagues demonstrated, by using high-resolution imaging approach, the intricate organization of cytoskeletal elements as neurofilaments and β-tubulin in close association to Ser129-phosphorylated α-synuclein at the peripheral portion of Lewy bodies (Moors et al., 2021). Indeed, these authors contributed towards the understanding of "orchestration of Lewy pathology in Parkinson's disease" and suggested that a regulated Lewy body morphogenesis and maturation take place in neurons starting from aggresomes. The formation of aggresomes is a strategy employed by the cells towards the clearance of ubiquitinated α-synuclein aggregates, which are too big to be degraded by the ubiquitin proteasome pathway (Bence et al., 2001). The aggresome formation starts at the microtubule organizing center (MTOC) and it is driven by HDAC6, which exerts its role through inherent modulatory effects on cytoskeletal proteins. As such, HDAC6 essentially behaves as a bridge between microtubule network and polyubiquitinated misfolded proteins. This is also the case for α-synuclein (Richter-Landsberg and Leyk, 2013). Furthermore, HDAC6 and its phosphorylated form have recently been localized in Lewy bodies and in glial cytoplasmic inclusions of multiple system atrophy (Mazzetti et al., 2020a). Indeed, aggresome could be considered an important defence mechanism recruited to counteract misfolded proteins accumulation for those aggregates that can be degraded solely by macroautophagy (Lamark and Johansen, 2010). Interestingly, it has been proposed that such mechanism could represent an initial step towards to Lewy body morphogenesis (Moors et al., 2021). Furthermore, evidence linked aggresomes to inflammasomes (Magupalli et al., 2020), a word coined in 2002 to define a multiprotein complex that play key roles in immune surveillance and mediates production and the rapid inflammatory form of cell death called "pyroptosis" (Broz and Dixit, 2016; Rathinam et al., 2012; Strowig et al., 2012). Inflammasome is also linked to microtubule cytoskeleton, due to its formation at the MTOC and the HDAC6 engagement. Furthermore, inflammasome degradation is induced by MTOC, promoting fusion of autophagosomes with lysosomes, enriched in the pericentriolar region (Kagan et al., 2014). Thus, MTOC seems to have a dual opposite role, either activating or inhibiting inflammasome. This link could also enforce the suggested interplay between inflammation and neurodegeneration, and in particular synucleinophaties, since inflammasome signalling was associated also to Parkinson's disease (Haque et al., 2020). Interestingly, α-synuclein aggregation has been shown to activate inflammasome trough microtubule-driven mechanisms in MPP^{+}-treated murine cells (Xueping et al., 2022).

Despite synucleinopathies and tauopathies exhibit different neuropathological features, several findings support the idea that they could share some of the mechanisms that lead to aggregation (Hamilton, 2000; Judkins et al., 2002; Lippa et al., 1998; Mori et al., 2002; Yancopoulou et al., 2005). The first key difference involves mutations in the *SNCA* and *MAPT* genes, encoding for α-synuclein and tau, respectively, which can both lead to parkinsonism-characterized neurodegenerative diseases (Dumanchin et al., 1998; Fujioka et al., 2014; Polymeropoulos et al., 1997; Spillantini et al., 1998). Secondly, α-synuclein and tau inclusions co-occur in multiple diseases, such as Alzheimer's disease with Lewy bodies, dementia with Lewy bodies and Parkinson's disease with dementia (Lippa et al., 1999; Lippa et al., 1998; Moussaud et al., 2014). Finally, phosphorylated tau is found in both Lewy bodies (Ishizawa et al., 2003) apart from neurofibrillary tangles, which are not exclusive for tauopathies but are frequently present also in Parkinson's disease patients (Galpern and Lang, 2006; Zhang et al., 2018). From a mechanistic point of view, evidence exists towards the proposed α-synuclein mediated tau pathology. We mentioned above that α-synuclein promotes tau phosphorylation and aggregation (Jensen et al., 1999). To note, α-synuclein and tau synergically promote the mutual polymerization into fibrils in multiple animal models including transgenic mice expressing A53T human α-synuclein and bigenic mice expressing wild-type human α-synuclein plus P301L mutant tau (Giasson et al., 2003).

6. Conclusion and final remarks

Albeit a vast literature addressed the mechanisms underlying neurodegeneration and proposed a role for a plethora of intracellular targets and pathways, many questions remain unanswered, and many knowledge gaps are still answering. Nonetheless, with respect to α-synuclein biology, here we propose a novel perspective towards the interpretation of the role α-synuclein holds in neurodegeneration and image a bunch of events whereby defects in the interplay between α-synuclein and tubulin/microtubules could initiate a pathogenic cascade leading to neuronal death (Fig. 1). Indeed, the deleterious impact resulting from the impaired cross-talk between α-synuclein and the microtubular cytoskeleton could include: change in microtubule stability, imbalance of post-translational modifications of tubulin, alteration of intraneuronal trafficking, dysregulation of synaptic activity, and, last but not least, α-synuclein aggregation.

How can the defective interaction between α-synuclein and tubulin/microtubules be explained? Intrinsic properties of α-synuclein have the potential to interfere and modulate α-synuclein and tubulin/microtubules interplay. Although the molecular details are yet to emerge, data from in cell-free systems suggest that point mutations related to Parkinson's disease compromise the ability of α-synuclein to interact with tubulin/microtubules (Cartelli et al., 2016; Toba et al., 2017). Conversely, microtubule or tubulin deficiency in binding α-synuclein could be the trigger for cellular dysfunctions. Microtubule alterations, as an example changes in the levels of their post-translational modifications, could prevent their interaction with α-synuclein. Additionally, if we imagine a microtubule as a track that is responsible for directed movement within the cells, the lacking interaction with α-synuclein could primarily provoke an impairment in its transport and prevent it from reaching its proper destination, the presynaptic compartment for instance. A similar effect could be attributed to chemical or functional modifications of microtubules themselves leading to defects of proteins that bind both microtubule and α-synuclein as, in this context, molecular motors could be. Hence, modulating or restoring the physiological interaction, be it directly and/or mediated, of α-synuclein with the microtubule system could be beneficial for correct neuronal activity. However, the challenge lies with the identification of how to achieve it. From a microtubule point of view, a multi-level strategy could rely on a pharmacological approach for correcting those microtubule defects that have been revealed by a large amount of *in vitro* and *in vivo* studies in models of neurodegeneration (Cappelletti et al., 2017). Among the others, strategies based on peptides that target microtubules could be promising. Interestingly, the peptide davunetide (NAP) stabilizes microtubules, promotes their assembly, thus reducing both tau phosphorylation (Magen et al., 2014) and α-synuclein aggregation (Fleming et al., 2011) in mouse model overexpressing α-synuclein. Likewise, in cybrid cell culture, NAP improves microtubule-dependent mitochondria traffic and leads to a decrease of α-synuclein oligomer content, thus recovering cell homeostasis (Esteves et al., 2014). In addition, SKIP, another peptide consisting of only 4 amino acids, that crosses the blood-brain barrier better than NAP, is able to interact with NAP motif in activity-dependent neuroprotective protein (ADNP) and to preserve microtubules (Ivashko-Pachima et al., 2021). Notably, SKIP (Ivashko-Pachima et al., 2021) and ADNP (Hadar et al., 2021) can repair microtubules dynamics, restoring axonal transport in rat model or mouse/human *in vitro* cell models of Parkinson's disease. All together these data clearly indicate that restoring or protecting microtubules impact on tau and α-synuclein aggregation and, consequently, on cell wellness. This has driven clinical trials in the field of tauopathies but, unfortunately, the results did not fit with the promising background (Tsai et al., 2020). From the side of α-synuclein, there is still a long way to go. With respect to α-synuclein biology, and more specifically on its interaction with tubulin/microtubules, a pharmacological approach could be a step further as, interestingly, investigated within an ongoing project funded by the European Community (http://www.tubintrain.eu).

Which are the current paradigms for understanding the roles of α-synuclein in the context of neurodegeneration? Two sides of the coin are worthy of consideration: the first is to place at the epicenter of research the idea that α-synuclein gains a toxic function and, in turn, leads to neurodegeneration, and on the opposite face we could suggest that the triggering force in neurodegeneration could be the loss of a physiologically active α-synuclein.

In respect to the first side, the issue of α-synuclein aggregation is central to synucleinopathies and is linked to the gain of a toxic function. Starting from monomeric and tetrameric α-synuclein, which are the physiological forms of the protein, multiple states of aggregation are described, which ultimately lead to the formation of the mature aggregates as Lewy bodies. A consensus has been achieved in the scientific community on the role of mature aggregates as scavengers that sequester toxic species. In this respect, the toxic species might be the early forms of aggregated α-synuclein as oligomers. Thus, the strategy of preventing their formation or sequestering these species has the potential to be the correct approach. Interestingly, among the players that could influence the aggregation processes there is the microtubule system (Alim et al., 2002; Esteves, 2010; Kim et al., 2008; Nakayama et al., 2012; Outeiro et al., 2007). This might suggest that targeting the interplay between α-synuclein and microtubules could prevent α-synuclein aggregation. To date, the evidence that aggregated α-synuclein is toxic established the theoretical background for the development of immunotherapy based on the use of antibodies that recognize and sequester aggregated form of α-synuclein. In this field, the work is in progress (Oliveira et al., 2021). Some promising results come from a clinical trial in early Parkinson's disease using the Prasinezumab (PASADENA, a Phase II clinical trial, Roche), a monoclonal antibody directed against an epitope in the C-terminus of α-synuclein and binding human aggregated α-synuclein with high affinity (Pagano et al., 2021). On the contrary, SPARK study, a Phase II clinical trial based on the monoclonal anti-pathological α-synuclein Cinpanemab (BIIB054) in early Parkinson's disease, did not meet its primary and secondary outcomes and was discontinued (https://ClinicalTrials.gov identifier NCT03318523).

The other side of the coin, however, suggests that the triggering force in neurodegeneration could be the loss of a physiologically active α-synuclein. According to this view, when α-synuclein cannot exert its function, due to its decreased levels or its defective nature, irreversible neuronal damage occurs. This is a change of the paradigm for which the pathological condition is a proteinopenia, instead of proteinopathy, as recently proposed for Alzheimer's and Parkinson's diseases (Espay, 2022a, 2022b; Espay et al., 2019). So, looking at the α-synuclein biology, the crucial step in triggering neuronal damage could be the loss of its physiological functions including vesicle formation (Burré et al., 2010), vesicle recycling and synaptic release (Nemani et al., 2010), interaction with lipids (Domingues et al., 2022) and, last but not least, interaction with microtubules (Cartelli et al., 2016).

The extraordinary complexity of normal and disease function of α-synuclein needs an extraordinary effort to fill the current gaps and translate the big amount of experimental data into a strategy for therapeutic intervention. Here, by collecting evidence and ideas on the emerging cross-talk between α-synuclein and tubulin/microtubules, we aimed to offer an additional brushstroke on the painting that, over time, will produce a coherent view of the pathogenetic mechanisms of neurodegeneration and, hopefully, will reveal tools to manage, mitigate and cure the disease.

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Declaration of Competing Interest

The authors reported no conflict of interest.

Data availability

Data will be made available on request.

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References

Aiken, J., Holzbaur, E.L.F., 2021. Cytoskeletal regulation guides neuronal trafficking to effectively supply the synapse. Curr. Biol. 31, R633–R650. https://doi.org/10.1016/j.cub.2021.02.024.

Alim, M.A., Hossain, M.S., Arima, K., Takeda, K., Izumiyama, Y., Nakamura, M., Kaji, H., Shinoda, T., Hisanaga, S., Uéda, K., 2002. Tubulin seeds α-synuclein fibril formation. J. Biol. Chem. 277, 2112–2117. https://doi.org/10.1074/jbc.M102981200.

Alim, M.A., Ma, Q.L., Takeda, K., Aizawa, T., Matsubara, M., Nakamura, M., Asada, A., Saito, T., Kaji, H., Yoshii, M., Hisanaga, S., Uéda, K., 2004. Demonstration of a role for α-synuclein as a functional microtubule-associated protein. J. Alzheimers Dis. 6, 435–442. https://doi.org/10.3233/JAD-2004-6412.

Amadeo, A., Pizzi, S., Comincini, A., Modena, D., Calogero, A.M., Madaschi, L., Faustini, G., Rolando, C., Bellucci, A., Pezzoli, G., Mazzetti, S., Cappelletti, G., 2021. The association between α-synuclein and α-tubulin in brain synapses. Int. J. Mol. Sci. 22 https://doi.org/10.3390/ijms22179153.

Anderson, P., Kedersha, N., 2002. &cestchinlong;Visibly stressed: the role of eIF2, TIA-1, and stress granules in protein translation. Cell Stress Chaperones 7, 213. https://doi.org/10.1379/1466-1268(2002)007<0213:VSTROE>2.0.CO;2.

Archer, S.L., 2013. Mitochondrial dynamics — mitochondrial fission and fusion in human diseases. N. Engl. J. Med. 369, 2236–2251. https://doi.org/10.1056/NEJMra1215233.

Baas, P.W., Qiang, L., 2019. Tau: It’s not what you think. Trends Cell Biol. 29, 452–461. https://doi.org/10.1016/j.tcb.2019.02.007.

Baas, P.W., Rao, A.N., Matamoros, A.J., Leo, L., 2016. Stability properties of neuronal microtubules. Cytoskeleton 73, 442–460. https://doi.org/10.1002/cm.21286.

Bence, N.F., Sampat, R.M., Kopito, R.R., 2001. Impairment of the ubiquitin-proteasome system by protein aggregation. Science 1979 (292), 1552–1555. https://doi.org/10.1126/science.292.5521.1552.

Bengoa-Vergniory, N., Roberts, R.F., Wade-Martins, R., Alegre-Abarrategui, J., 2017. Alpha-synuclein oligomers: a new hope. Acta Neuropathol. 134, 819–838. https://doi.org/10.1007/s00401-017-1755-1.

Bertoncini, C.W., Jung, Y.-S., Fernandez, C.O., Hoyer, W., Griesinger, C., Jovin, T.M., Zweckstetter, M., 2005. From the cover: release of long-range tertiary interactions potentiates aggregation of natively unstructured -synuclein. Proc. Natl. Acad. Sci. 102, 1430–1435. https://doi.org/10.1073/pnas.0407146102.

Betzer, C., Movius, A.J., Shi, M., Gai, W.-P., Zhang, J., Jensen, P.H., 2015. Identification of Synaptosomal proteins binding to monomeric and oligomeric α-Synuclein. PLoS One 10, e0116473. https://doi.org/10.1371/journal.pone.0116473.

Bodaleo, F.J., Gonzalez-Billault, C., 2016. The presynaptic microtubule cytoskeleton in physiological and pathological conditions: lessons from Drosophila fragile X syndrome and hereditary spastic paraplegias. Front. Mol. Neurosci. 9, 1–16. https://doi.org/10.3389/fnmol.2016.00060.

Brandt, R., Bakota, L., 2017. Microtubule dynamics and the neurodegenerative triad of Alzheimer’s disease: the hidden connection. J. Neurochem. 143, 409–417. https://doi.org/10.1111/jnc.14011.

Broz, P., Dixit, V.M., 2016. Inflammasomes: mechanism of assembly, regulation and signalling. Nat. Rev. Immunol. 16, 407–420. https://doi.org/10.1038/nri.2016.58.

Burré, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M.R., Südhof, T.C., 2010. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 329, 1663–1667. https://doi.org/10.1126/science.1195227.

Burré, J., Vivona, S., Diao, J., Sharma, M., Brunger, A.T., Südhof, T.C., 2013. Properties of native brain α-synuclein. Nature 498, E4–E6. https://doi.org/10.1038/nature12125.

Burré, J., Sharma, M., Südhof, T.C., 2018. Cell biology and pathophysiology of α-Synuclein. Cold Spring Harb Perspect Med 8, a024091. https://doi.org/10.1101/cshperspect.a024091.

Calogero, A.M., Mazzetti, S., Pezzoli, G., Cappelletti, G., 2019. Neuronal microtubules and proteins linked to Parkinson’s disease: A relevant interaction? Biol. Chem. 400, 1099–1112. https://doi.org/10.1515/hsz-2019-0142.

Cappelletti, G., Cartelli, D., Christodoulou, M., Passarella, D., 2017. Microtubule-directed therapeutic strategy for neurodegenerative disorders: starting from the basis and looking on the emergences. Curr. Pharm. Des. 23, 784–808. https://doi.org/10.2174/1381612822666161214150544.

Cappelletti, G., Calogero, A.M., Rolando, C., 2021. Microtubule acetylation: A reading key to neural physiology and degeneration. Neurosci. Lett. 755, 135900 https://doi.org/10.1016/j.neulet.2021.135900.

Carnwath, T., Mohammed, R., Tsiang, D., 2018. The direct and indirect effects of α-synuclein on microtubule stability in the pathogenesis of Parkinson’s disease. Neuropsychiatr. Dis. Treat. 14, 1685–1695. https://doi.org/10.2147/NDT.S166322.

Cartelli, D., Cappelletti, G., 2017. Microtubule destabilization paves the way to Parkinson’s disease. Mol. Neurobiol. 54, 6762–6774. https://doi.org/10.1007/s12035-016-0188-5.

Cartelli, D., Aliverti, A., Barbiroli, A., Santambrogio, C., Ragg, E.M., Casagrande, F.V.M., Cantele, F., Beltramone, S., Marangon, J., De Gregorio, C., Pandini, V., Emanuele, M., Chieregatti, E., Pieraccini, S., Holmqvist, S., Bubacco, L., Roybon, L., Pezzoli, G., Grandori, R., Arnal, I., Cappelletti, G., 2016. α-Synuclein is a novel microtubule Dynamase. Sci. Rep. 6, 1–13. https://doi.org/10.1038/srep33289.

Chambraud, B., Byrne, C., Meduri, G., Baulieu, E.E., Giustiniani, J., 2022. FKBP52 in neuronal signaling and neurodegenerative diseases: A microtubule story. Int. J. Mol. Sci. 23, 1738. https://doi.org/10.3390/ijms23031738.

Dedmon, M.M., Lindorff-Larsen, K., Christodoulou, J., Vendruscolo, M., Dobson, C.M., 2005. Mapping long-range interactions in α-Synuclein using spin-label NMR and ensemble molecular dynamics simulations. J. Am. Chem. Soc. 127, 476–477. https://doi.org/10.1021/ja044834j.

Dent, E.W., 2020. Dynamic microtubules at the synapse. Curr. Opin. Neurobiol. 63, 9–14. https://doi.org/10.1016/j.conb.2020.01.003.

Devi, L., Raghavendran, V., Prabhu, B.M., Avadhani, N.G., Anandatheerthavarada, H.K., 2008. Mitochondrial import and accumulation of α-Synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. J. Biol. Chem. 283, 9089–9100. https://doi.org/10.1074/jbc.M710012200.

Dickson, D.W., 2012. Parkinson’s disease and parkinsonism: neuropathology. Cold Spring Harb Perspect Med 2, a009258. https://doi.org/10.1101/cshperspect.a009258.

Domingues, R., Sant’Anna, R., da Fonseca, A.C.C., Robbs, B.K., Foguel, D., Outeiro, T.F., 2022. Extracellular alpha-synuclein: sensors, receptors, and responses. Neurobiol. Dis. 168, 105696 https://doi.org/10.1016/j.nbd.2022.105696.

Donadio, V., Incensi, A., Cortelli, P., Giannoccaro, M.P., Jaber, M.A., Baruzzi, A., Liguori, R., 2013. Skin sympathetic fiber alpha-synuclein deposits: A potential biomarker for pure autonomic failure. Neurology 80, 725–732. https://doi.org/10.1212/WNL.0b013e3182825127.

Duka, T., Duka, V., Joyce, J.N., Sidhu, A., 2009. α-Synuclein contributes to GSK-3β-catalyzed tau phosphorylation in Parkinson’s disease models. FASEB J. 23, 2820–2830. https://doi.org/10.1096/fj.08-120410.

Dumanchin, C., Camuzat, A., Campion, D., Verpillat, P., Hannequin, D., Dubois, B., Saugier-Veber, P., Martin, C., Penet, C., Charbonnier, F., Agid, Y., Frebourg, T., Brice, A., 1998. Segregation of a missense mutation in the microtubule-associated protein tau gene with familial frontotemporal dementia and parkinsonism. Hum. Mol. Genet. 7, 1825–1829. https://doi.org/10.1093/hmg/7.11.1825.

d’Ydewalle, C., Krishnan, J., Chiheb, D.M., Van Damme, P., Irobi, J., Kozikowski, A.P., Vanden Berghe, P., Timmerman, V., Robberecht, W., Van Den Bosch, L., 2011. HDAC6 inhibitors reverse axonal loss in a mouse model of mutant HSPB1–induced Charcot-Marie-tooth disease. Nat. Med. 17, 968–974. https://doi.org/10.1038/nm.2396.

Eguchi, K., Taoufiq, Z., Thorn-Seshold, O., Trauner, D., Hasegawa, M., Takahashi, T., 2017. Wild-type monomeric α-synuclein can impair vesicle endocytosis and synaptic fidelity via tubulin polymerization at the calyx of held. J. Neurosci. 37, 6043–6052. https://doi.org/10.1523/JNEUROSCI.0179-17.2017.

Espay, A.J., 2022a. Synuclein Aggregation Depletes Normal Synuclein: From Proteinopathy to Proteinopenia., in: Synuclein Meeting, 12-15 April 2022, Leuven. Sissa Medialab, Belgium, p. 60.

Espay, A.J., 2022b. Movement disorders research in 2021: cracking the paradigm. Lancet Neurol. 21, 10–11. https://doi.org/10.1016/S1474-4422(21)00413-0.

Espay, A.J., Vizcarra, J.A., Marsili, L., Lang, A.E., Simon, D.K., Merola, A., Josephs, K.A., Fasano, A., Morgante, F., Savica, R., Greenamyre, J.T., Cambi, F., Yamasaki, T.R., Tanner, C.M., Gan-Or, Z., Litvan, I., Mata, I.F., Zabetian, C.P., Brundin, P., Fernandez, H.H., Standaert, D.G., Kauffman, M.A., Schwarzschild, M.A., Sardi, S.P., Sherer, T., Perry, G., Leverenz, J.B., 2019. Revisiting protein aggregation as pathogenic in sporadic Parkinson and Alzheimer diseases. Neurology 92, 329–337. https://doi.org/10.1212/WNL.0000000000006926.

Esteves, A.R., 2010. Microtubule depolymerization potentiates alpha-synuclein oligomerization. Front. Aging Neurosci. 1, 1–6. https://doi.org/10.3389/neuro.24.005.2009.

Esteves, A.R., Gozes, I., Cardoso, S.M., 2014. The rescue of microtubule-dependent traffic recovers mitochondrial function in Parkinson’s disease. Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1842, 7–21. https://doi.org/10.1016/j.bbadis.2013.10.003.

Fleming, S.M., Mulligan, C.K., Richter, F., Mortazavi, F., Lemesre, V., Frias, C., Zhu, C., Stewart, A., Gozes, I., Morimoto, B., Chesselet, M.-F., 2011. A pilot trial of the microtubule-interacting peptide (NAP) in mice overexpressing alpha-synuclein shows improvement in motor function and reduction of alpha-synuclein inclusions. Mol. Cell. Neurosci. 46, 597–606. https://doi.org/10.1016/j.mcn.2010.12.011.

Fujioka, S., Ogaki, K., Tacik, P.M., Uitti, R.J., Ross, O.A., Wszolek, Z.K., 2014. Update on novel familial forms of Parkinson's disease and multiple system atrophy. Parkinsonism Relat. Disord. 20, S29–S34. https://doi.org/10.1016/S1353-8020(13)70010-5.

Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M.S., Shen, J., Takio, K., Iwatsubo, T., 2002. α-Synuclein is phosphorylated in synucleinopathy lesions. Nat. Cell Biol. 4, 160–164. https://doi.org/10.1038/ncb748.

Galloway, P.G., Mulvihill, P., Perry, G., 1992. Filaments of Lewy bodies contain insoluble cytoskeletal elements. Am. J. Pathol. 140, 809–822.

Galpern, W.R., Lang, A.E., 2006. Interface between tauopathies and synucleinopathies: A tale of two proteins. Ann. Neurol. 59, 449–458. https://doi.org/10.1002/ana.20819.

George, J.M., Jin, H., Woods, W.S., Clayton, D.F., 1995. Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. Neuron 15, 361–372. https://doi.org/10.1016/0896-6273(95)90040-3.

Gerard, M., Deleersnijder, A., Daniels, V., Schreurs, S., Munck, S., Reumers, V., Pottel, H., Engelborghs, Y., van den Haute, C., Taymans, J.-M., Debyser, Z., Baekelandt, V., 2010. Inhibition of FK506 binding proteins reduces -Synuclein aggregation and Parkinson's disease-like pathology. J. Neurosci. 30, 2454–2463. https://doi.org/10.1523/JNEUROSCI.5983-09.2010.

Giasson, B.I., Forman, M.S., Higuchi, M., Golbe, L.I., Graves, C.L., Kotzbauer, P.T., Trojanowski, J.Q., Lee, V.M.Y., 2003. Initiation and synergistic fibrillization of tau and alpha-Synuclein. Science 1979 (300), 636–640. https://doi.org/10.1126/science.1082324.

Goedert, M., Jakes, R., Spillantini, M.G., 2017. The Synucleinopathies: twenty years on. J. Parkinsons Dis. 7, S51–S69. https://doi.org/10.3233/JPD-179005.

Gordon-Weeks, P.R., Burgoyne, R.D., Gray, E.G., 1982. Presynaptic microtubules: organization and assembly/disassembly. Neuroscience 7, 739–749.

Guedes-Dias, P., Holzbaur, E.L.F., 2019. Axonal transport: driving synaptic function. Science (1979) 366, 1–35. https://doi.org/10.1126/science.aaw9997.

Hadar, A., Kapitansky, O., Ganaiem, M., Sragovich, S., Lobyntseva, A., Giladi, E., Yeheskel, A., Avitan, A., Vatine, G.D., Gurwitz, D., Ivashko-Pachima, Y., Gozes, I., 2021. Introducing ADNP and SIRT1 as new partners regulating microtubules and histone methylation. Mol. Psychiatry 26, 6550–6561. https://doi.org/10.1038/s41380-021-01143-9.

Haïk, S., Privat, N., Adjou, K.T., Sazdovitch, V., Dormont, D., Duyckaerts, C., Hauw, J.J., 2002. Alpha-synuclein-immunoreactive deposits in human and animal prion diseases. Acta Neuropathol. 103, 516–520. https://doi.org/10.1007/s00401-001-0499-z.

Hamilton, R.L., 2000. Lewy bodies in Alzheimer's disease: A neuropathological review of 145 cases using α-Synuclein immunohistochemistry. Brain Pathol. 10, 378–384. https://doi.org/10.1111/j.1750-3639.2000.tb00269.x.

Haque, M.E., Akther, M., Jakaria, M., Kim, I., Azam, S., Choi, D., 2020. Targeting the microglial NLRP3 Inflammasome and its role in Parkinson's disease. Mov. Disord. 35, 20–33. https://doi.org/10.1002/mds.27874.

Hirsch, E.C., Hunot, S., 2009. Neuroinflammation in Parkinson's disease: a target for neuroprotection? Lancet Neurol. 8, 382–397. https://doi.org/10.1016/S1474-4422(09)70062-6.

Holec, S.A.M., Liu, S.L., Woerman, A.L., 2022. Consequences of variability in α-synuclein fibril structure on strain biology. Acta Neuropathol. 143, 311–330. https://doi.org/10.1007/s00401-022-02403-w.

Holmes, B.B., Furman, J.L., Mahan, T.E., Yamasaki, T.R., Mirbaha, H., Eades, W.C., Belaygorod, L., Cairns, N.J., Holtzman, D.M., Diamond, M.I., 2014. Proteopathic tau seeding predicts tauopathy in vivo. Proc. Natl. Acad. Sci. 111, E4376–E4385. https://doi.org/10.1073/pnas.1411649111.

Hussein, A.M., Eldosoky, M., El-Shafey, M., El-Mesery, M., Ali, A.N., Abbas, K.M., Abulseoud, O.A., 2019. Effects of metformin on apoptosis and α-synuclein in a rat model of pentylenetetrazole-induced epilepsy. Can. J. Physiol. Pharmacol. 97, 37–46. https://doi.org/10.1139/cjpp-2018-0266.

Iseki, E., Marui, W., Sawada, H., Uéda, K., Kosaka, K., 2000. Accumulation of human α-synuclein in different cytoskeletons in Lewy bodies in brains of dementia with Lewy bodies. Neurosci. Lett. 290, 41–44. https://doi.org/10.1016/S0304-3940(00)01316-1.

Ishizawa, T., Mattila, P., Davies, P., Wang, D., Dickson, D.W., 2003. Colocalization of tau and alpha-Synuclein epitopes in Lewy bodies. J. Neuropathol. Exp. Neurol. 62, 389–397. https://doi.org/10.1093/jnen/62.4.389.

Ito, H., Nakayama, K., Jin, C., Suzuki, Y., Yazawa, I., 2012. α-Synuclein accumulation reduces GABAergic inhibitory transmission in a model of multiple system atrophy. Biochem. Biophys. Res. Commun. 428, 348–353. https://doi.org/10.1016/j.bbrc.2012.10.057.

Ivashko-Pachima, Y., Seroogy, K.B., Sharabi, Y., Gozes, I., 2021. Parkinson disease-modification encompassing rotenone and 6-Hydroxydopamine neurotoxicity by the microtubule-protecting drug candidate SKIP. J. Mol. Neurosci. 71, 1515–1524. https://doi.org/10.1007/s12031-021-01876-w.

Janke, C., Magiera, M.M., 2020. The tubulin code and its role in controlling microtubule properties and functions. Nat. Rev. Mol. Cell Biol. 21, 307–326. https://doi.org/10.1038/s41580-020-0214-3.

Jensen, P.H., Hager, H., Nielsen, M.S., Højrup, P., Gliemann, J., Jakes, R., 1999. α-Synuclein binds to tau and stimulates the protein kinase A-catalyzed tau phosphorylation of serine residues 262 and 356. J. Biol. Chem. 274, 25481–25489. https://doi.org/10.1074/jbc.274.36.25481.

Jensen, P.H., Islam, K., Kenney, J., Nielsen, M.S., Power, J., Gai, W.P., 2000. Microtubule-associated protein 1B is a component of cortical Lewy bodies and binds α-Synuclein filaments. J. Biol. Chem. 275, 21500–21507. https://doi.org/10.1074/jbc.M000099200.

Judkins, A., Forman, M., Uryu, K., Hinkle, D., Asbury, A., Lee, V., Trojanowski, J., 2002. Co-occurrence of Parkinson's disease with progressive supranuclear palsy. Acta Neuropathol. 103, 526–530. https://doi.org/10.1007/s00401-001-0483-7.

Kagan, J.C., Magupalli, V.G., Wu, H., 2014. SMOCs: supramolecular organizing centres that control innate immunity. Nat. Rev. Immunol. 14, 821–826. https://doi.org/10.1038/nri3757.

Kapitein, L.C., Hoogenraad, C.C., 2015. Building the neuronal microtubule cytoskeleton. Neuron 87, 492–506. https://doi.org/10.1016/j.neuron.2015.05.046.

Kilpeläinen, T.P., Hellinen, L., Vrijdag, J., Yan, X., Svarcbahs, R., Vellonen, K.-S., Lambeir, A.-M., Huttunen, H., Urtti, A., Wallen, E.A.A., Myöhänen, T.T., 2020. The effect of prolyl oligopeptidase inhibitors on alpha-synuclein aggregation and autophagy cannot be predicted by their inhibitory efficacy. Biomed. Pharmacother. 128, 110253 https://doi.org/10.1016/j.biopha.2020.110253.

Kim, M., Jung, W., Lee, I.H., Bhak, G., Paik, S.R., Hahn, J.S., 2008. Impairment of microtubule system increases α-synuclein aggregation and toxicity. Biochem. Biophys. Res. Commun. 365, 628–635. https://doi.org/10.1016/j.bbrc.2007.11.020.

Koga, S., Lin, W.L., Walton, R.L., Ross, O.A., Dickson, D.W., 2018. TDP-43 pathology in multiple system atrophy: colocalization of TDP-43 and α-synuclein in glial cytoplasmic inclusions. Neuropathol. Appl. Neurobiol. 44, 707–721. https://doi.org/10.1111/nan.12485.

Lamark, T., Johansen, T., 2010. Autophagy: links with the proteasome. Curr. Opin. Cell Biol. 22, 192–198. https://doi.org/10.1016/j.ceb.2009.11.002.

Lassen, L.B., Reimer, L., Ferreira, N., Betzer, C., Jensen, P.H., 2016. Protein partners of α-Synuclein in health and disease. Brain Pathol. 26, 389–397. https://doi.org/10.1111/bpa.12374.

Lee, H.-J., Khoshaghideh, F., Lee, S., Lee, S.-J., 2006. Impairment of microtubule-dependent trafficking by overexpression of α-synuclein. Eur. J. Neurosci. 24, 3153–3162. https://doi.org/10.1111/j.1460-9568.2006.05210.x.

Lehotzky, A., Tirián, L., Tökési, N., Lénárt, P., Szabó, B., Kovács, J., Ovádi, J., 2004. Dynamic targeting of microtubules by TPPP/p25 affects cell survival. J. Cell Sci. 117, 6249–6259. https://doi.org/10.1242/jcs.01550.

Lemos, M., Stefanova, N., 2020. Histone deacetylase 6 and the disease mechanisms of α-Synucleinopathies. Front Synaptic Neurosci 12, 1–13. https://doi.org/10.3389/fnsyn.2020.586453.

Lippa, C.F., Fujiwara, H., Mann, D.M.A., Giasson, B., Baba, M., Schmidt, M.L., Nee, L.E., Connell, B.O., Pollen, D.A., George-hyslop, P.S., Ghetti, B., Nochlin, D., Bird, T.D., Cairns, N.J., Lee, V.M., Iwatsubo, T., Trojanowski, J.Q., 1998. Lewy bodies contain altered alpha-Synuclein in brains of many familial Alzheimer ' s disease patients with mutations in Presenilin and amyloid precursor protein genes. Am. J. Pathol. 153, 1365–1370.

Lippa, C.F., Schmidt, M.L., Lee, V.M.Y., Trojanowski, J.Q., 1999. Antibodies to alpha-synuclein detect Lewy bodies in many Down's syndrome brains with Alzheimer's disease. Ann. Neurol. 45, 353–357. https://doi.org/10.1002/1531-8249(199903)45:3<353::AID-ANA11>3.0.CO;2-4.

Magen, I., Ostritsky, R., Richter, F., Zhu, C., Fleming, S.M., Lemesre, V., Stewart, A.J., Morimoto, B.H., Gozes, I., Chesselet, M., 2014. Intranasal NAP (davunetide) decreases tau hyperphosphorylation and moderately improves behavioral deficits in mice overexpressing α-synuclein. Pharmacol. Res. Perspect. 2, 1–20. https://doi.org/10.1002/prp2.65.

Magupalli, V.G., Negro, R., Tian, Y., Hauenstein, A., Caprio, G., Skillern, W., Deng, Q., Orning, P., Hasan, B., Maliga, Z., Sharif, H., Hu, J.J., Evavold, C.L., Kagan, C., Schmidt, F.I., Fitzgerald, K.A., Kirchhausen, T., 2020. HDAC6 mediates an aggresome-like mechanism for NLRP3 and pyrin inflammasome activation. Science 1979 (369), 1–36. https://doi.org/10.1126/science.aas8995.HDAC6.

Matamoros, A.J., Baas, P.W., 2016. Microtubules in health and degenerative disease of the nervous system. Brain Res. Bull. 126, 217–225. https://doi.org/10.1016/j.brainresbull.2016.06.016.

Mazzetti, S., Basellini, M.J., Ferri, V., Cassani, E., Cereda, E., Paolini, M., Calogero, A.M., Bolliri, C., De Leonardis, M., Sacilotto, G., Cilia, R., Cappelletti, G., Pezzoli, G., 2020a. α-Synuclein oligomers in skin biopsy of idiopathic and monozygotic twin patients with Parkinson's disease. Brain 143. https://doi.org/10.1093/brain/awaa008.

Mazzetti, S., de Leonardis, M., Gagliardi, G., Calogero, A.M., Basellini, M.J., Madaschi, L., Costa, I., Cacciatore, F., Spinello, S., Bramerio, M., Cilia, R., Rolando, C., Giaccone, G., Pezzoli, G., Cappelletti, G., 2020b. Phospho-HDAC6 gathers into protein aggregates in Parkinson's disease and atypical Parkinsonisms. Front. Neurosci. 14, 1–11. https://doi.org/10.3389/fnins.2020.00624.

Mazzetti, S., Barichella, M., Giampietro, F., Giana, A., Calogero, A.M., Amadeo, A., Palazzi, N., Comincini, A., Giaccone, G., Bramerio, M., Caronni, S., Cereda, V., Cereda, E., Cappelletti, G., Rolando, C., Pezzoli, G., 2022. Astrocytes expressing vitamin D-activating enzyme identify Parkinson's disease. CNS Neurosci Ther 1–11. https://doi.org/10.1111/cns.13801.

Moors, T.E., Maat, C.A., Niedieker, D., Mona, D., Petersen, D., Timmermans-Huisman, E., Kole, J., El-Mashtoly, S.F., Spycher, L., Zago, W., Barbour, R., Mundigl, O., Kaluza, K., Huber, S., Hug, M.N., Kremer, T., Ritter, M., Dziadek, S., Geurts, J.J.G., Gerwert, K., Britschgi, M., van de Berg, W.D.J., 2021. The subcellular arrangement of alpha-synuclein proteoforms in the Parkinson's disease brain as revealed by multicolor STED microscopy. Acta Neuropathol. 142, 423–448. https://doi.org/10.1007/s00401-021-02329-9.

Morato Torres, C.A., Wassouf, Z., Zafar, F., Sastre, D., Outeiro, T.F., Schüle, B., 2020. The role of alpha-Synuclein and other Parkinson's genes in neurodevelopmental and neurodegenerative disorders. Int. J. Mol. Sci. 21, 5724. https://doi.org/10.3390/ijms21165724.

Mori, H., Oda, M., Komori, T., Arai, N., Takanashi, M., Mizutani, T., Hirai, S., Mizuno, Y., 2002. Lewy bodies in progressive supranuclear palsy. Acta Neuropathol. 104, 273–278. https://doi.org/10.1007/s00401-002-0555-3.

Mosharov, E.V., Larsen, K.E., Kanter, E., Phillips, K.A., Schmitz, Y., Krantz, D.E., Kobayashi, K., Edwards, R.H., Sulzer, D., 2009. Interplay between cytosolic dopamine, calcium and α-synuclein causes selective death of substantia nigra neurons, 62, 218–229. https://doi.org/10.1016/j.neuron.2009.01.033.Interplay.

Moussaud, S., Jones, D.R., Moussaud-Lamodière, E.L., Delenclos, M., Ross, O.A., McLean, P.J., 2014. Alpha-synuclein and tau: teammates in neurodegeneration? Mol. Neurodegener. 9, 43. https://doi.org/10.1186/1750-1326-9-43.

Moutin, M., Bosc, C., Peris, L., Andrieux, A., 2021. Tubulin post-translational modifications control neuronal development and functions. Dev Neurobiol 81, 253–272. https://doi.org/10.1002/dneu.22774.

Nakayama, K., Suzuki, Y., Yazawa, I., 2009. Microtubule Depolymerization suppresses α-Synuclein accumulation in a mouse model of multiple system atrophy. Am. J. Pathol. 174, 1471–1480. https://doi.org/10.2353/ajpath.2009.080503.

Nakayama, K., Suzuki, Y., Yazawa, I., 2012. Binding of neuronal α-synuclein to β-III tubulin and accumulation in a model of multiple system atrophy. Biochem. Biophys. Res. Commun. 417, 1170–1175. https://doi.org/10.1016/j.bbrc.2011.12.092.

Nemani, V.M., Lu, W., Berge, V., Nakamura, K., Onoa, B., Lee, M.K., Chaudhry, F.A., Nicoll, R.A., Edwards, R.H., 2010. Increased expression of α-Synuclein reduces neurotransmitter release by inhibiting synaptic vesicle Reclustering after endocytosis. Neuron 65, 66–79. https://doi.org/10.1016/j.neuron.2009.12.023.

Oláh, J., Lehotzky, A., Szunyogh, S., Szénási, T., Orosz, F., Ovádi, J., 2020. Microtubule-associated proteins with regulatory functions by day and pathological potency at night. Cells 9, 357. https://doi.org/10.3390/cells9020357.

Olanow, C.W., Perl, D.P., DeMartino, G.N., McNaught, K.S.P., 2004. Lewy-body formation is an aggresome-related process: a hypothesis. Lancet Neurol. 3, 496–503. https://doi.org/10.1016/S1474-4422(04)00827-0.

Oliveira, L.M.A., Gasser, T., Edwards, R., Zweckstetter, M., Melki, R., Stefanis, L., Lashuel, H.A., Sulzer, D., Vekrellis, K., Halliday, G.M., Tomlinson, J.J., Schlossmacher, M., Jensen, P.H., Schulze-Hentrich, J., Riess, O., Hirst, W.D., El-Agnaf, O., Mollenhauer, B., Lansbury, P., Outeiro, T.F., 2021. Alpha-synuclein research: defining strategic moves in the battle against Parkinson's disease. NPJ Parkinsons Dis 7. https://doi.org/10.1038/s41531-021-00203-9.

Outeiro, T.F., Kontopoulos, E., Altmann, S.M., Kufareva, I., Strathearn, K.E., Amore, A. M., Volk, C.B., Maxwell, M.M., Rochet, J.-C., McLean, P.J., Young, A.B., Abagyan, R., Feany, M.B., Hyman, B.T., Kazantsev, A.G., 2007. Sirtuin 2 inhibitors rescue α-Synuclein-mediated toxicity in models of Parkinson's disease. Science 1979 (317), 516–519. https://doi.org/10.1126/science.1143780.

Pagano, G., Boess, F.G., Taylor, K.I., Ricci, B., Mollenhauer, B., Poewe, W., Boulay, A., Anzures-Cabrera, J., Vogt, A., Marchesi, M., Post, A., Nikolcheva, T., Kinney, G.G., Zago, W.M., Ness, D.K., Svoboda, H., Britschgi, M., Ostrowitzki, S., Simuni, T., Marek, K., Koller, M., Sevigny, J., Doody, R., Fontoura, P., Umbricht, D., Bonni, A., 2021. A phase II study to evaluate the safety and efficacy of Prasinezumab in early Parkinson's disease (PASADENA): rationale, design, and baseline data. Front. Neurol. 12, 1–17. https://doi.org/10.3389/fneur.2021.705407.

Pan, T., Kondo, S., Le, W., Jankovic, J., 2008. The role of autophagy-lysosome pathway in neurodegeneration associated with Parkinson's disease. Brain 131, 1969–1978. https://doi.org/10.1093/brain/awm318.

Panicker, N., Ge, P., Dawson, V.L., Dawson, T.M., 2021. The cell biology of Parkinson's disease. J. Cell Biol. 220, 621–631. https://doi.org/10.1083/jcb.202012095.

Payton, J.E., Perrin, R.J., Clayton, D.F., George, J.M., 2001. Protein–protein interactions of alpha-synuclein in brain homogenates and transfected cells. Mol. Brain Res. 95, 138–145. https://doi.org/10.1016/S0169-328X(01)00257-1.

Pellegrini, L., Wetzel, A., Grannó, S., Heaton, G., Harvey, K., 2017. Back to the tubule: microtubule dynamics in Parkinson's disease. Cell. Mol. Life Sci. 74, 409–434. https://doi.org/10.1007/s00018-016-2351-6.

Polymeropoulos, Mihael H., Lavedan, C., Leroy, E., Ide, S.E., Dehejia, A., Dutra, A., Pike, B., Root, H., Rubenstein, J., Boyer, R., Stenroos, E.S., Chandrasekharappa, S., Athanassiadou, A., Papapetropoulos, T., Johnson, W.G., Lazzarini, A.M., Duvoisin, R. C., di Iorio, G., Golbe, L.I., Nussbaum, R.L., 1997. Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science 1979 (276), 2045–2047. https://doi.org/10.1126/science.276.5321.2045.

Qu, X., Kumar, A., Blockus, H., Waites, C., Bartolini, F., 2019. Activity-dependent nucleation of dynamic microtubules at presynaptic boutons controls neurotransmission. Curr. Biol. 29, 4231–4240.e5. https://doi.org/10.1016/j.cub.2019.10.049.

Ransohoff, R.M., 2016. How neuroinflammation contributes to neurodegeneration. Science (1979) 353, 777–783. https://doi.org/10.1126/science.aag2590.

Rathinam, V.A.K., Vanaja, S.K., Fitzgerald, K.A., 2012. Regulation of inflammasome signaling. Nat. Immunol. 13, 333–342. https://doi.org/10.1038/ni.2237.

Richter-Landsberg, C., Leyk, J., 2013. Inclusion body formation, macroautophagy, and the role of HDAC6 in neurodegeneration. Acta Neuropathol. 126, 793–807. https://doi.org/10.1007/s00401-013-1158-x.

Roberts, R.F., Wade-Martins, R., Alegre-Abarrategui, J., 2015. Direct visualization of alpha-synuclein oligomers reveals previously undetected pathology in Parkinson's disease brain. Brain 138, 1642–1657. https://doi.org/10.1093/brain/awv040.

Roll-Mecak, A., Vale, R.D., 2008. Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin. Nature 451, 363–367. https://doi.org/10.1038/nature06482.

Rolls, M.M., Thyagarajan, P., Feng, C., 2021. Microtubule dynamics in healthy and injured neurons. Dev Neurobiol 81, 321–332. https://doi.org/10.1002/dneu.22746.

Salemi, M., Mazzetti, S., De Leonardis, M., Giampietro, F., Medici, V., Poloni, T.E., Cannarella, R., Giaccone, G., Pezzoli, G., Cappelletti, G., Ferri, R., 2021. Poly (ADP-ribose) polymerase 1 and Parkinson's disease: A study in post-mortem human brain. Neurochem. Int. 144, 104978 https://doi.org/10.1016/j.neuint.2021.104978.

Savolainen, M.H., Yan, X., Myöhänen, T.T., Huttunen, H.J., 2015. Prolyl oligopeptidase enhances α-Synuclein dimerization via direct protein-protein interaction. J. Biol. Chem. 290, 5117–5126. https://doi.org/10.1074/jbc.M114.592931.

Schapira, A.H.V., 2007. Mitochondrial dysfunction in Parkinson's disease. Cell Death Differ. 14, 1261–1266. https://doi.org/10.1038/sj.cdd.4402160.

Schulz, I., Zeitschel, U., Rudolph, T., Ruiz-Carrillo, D., Rahfeld, J.-U., Gerhartz, B., Bigl, V., Demuth, H.-U., Roßner, S., 2005. Subcellular localization suggests novel functions for prolyl endopeptidase in protein secretion. J. Neurochem. 94, 970–979. https://doi.org/10.1111/j.1471-4159.2005.03237.x.

Scott, D., Roy, S., 2012. Alpha-synuclein inhibits intersynaptic vesicle mobility and maintains recycling-Pool homeostasis. J. Neurosci. 32, 10129–10135. https://doi.org/10.1523/JNEUROSCI.0535-12.2012.

Seebauer, L., Schneider, Y., Drobny, A., Plötz, S., Koudelka, T., Tholey, A., Prots, I., Winner, B., Zunke, F., Winkler, J., Xiang, W., 2022. Interaction of alpha Synuclein and microtubule organization is linked to impaired Neuritic integrity in Parkinson's patient-derived neuronal cells. Int. J. Mol. Sci. 23, 1812. https://doi.org/10.3390/ijms23031812.

Sekiya, H., Kowa, H., Koga, H., Takata, M., Satake, W., Futamura, N., Funakawa, I., Jinnai, K., Takahashi, M., Kondo, T., Ueno, Y., Kanagawa, M., Kobayashi, K., Toda, T., 2019. Wide distribution of alpha-synuclein oligomers in multiple system atrophy brain detected by proximity ligation. Acta Neuropathol. 137, 455–466. https://doi.org/10.1007/s00401-019-01961-w.

Spillantini, M.G., Schmidt, M.L., Lee, V.M.-Y., Trojanowski, J.Q., Jakes, R., Goedert, M., 1997. α-Synuclein in Lewy bodies. Nature 388, 839–840. https://doi.org/10.1038/42166.

Spillantini, M.G., Murrell, J.R., Goedert, M., Farlow, M.R., Klug, A., Ghetti, B., 1998. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. Proc. Natl. Acad. Sci. U. S. A. 95, 7737–7741. https://doi.org/10.1073/pnas.95.13.7737.

Strowig, T., Henao-Mejia, J., Elinav, E., Flavell, R., 2012. Inflammasomes in health and disease. Nature 481, 278–286. https://doi.org/10.1038/nature10759.

Surmeier, D.J., Schumacker, P.T., Guzman, J.D., Ilijic, E., Yang, B., Zampese, E., 2017. Calcium and Parkinson's disease. Biochem. Biophys. Res. Commun. 483, 1013–1019. https://doi.org/10.1016/j.bbrc.2016.08.168.

Suzuki, Y., Jin, C., Iwase, T., Yazawa, I., 2014. β-III tubulin fragments inhibit α-synuclein accumulation in models of multiple system atrophy. J. Biol. Chem. 289, 24374–24382. https://doi.org/10.1074/jbc.M114.557215.

Swirski, M., Miners, J.S., de Silva, R., Lashley, T., Ling, H., Holton, J., Revesz, T., Love, S., 2014. Evaluating the relationship between amyloid-β and α-synuclein phosphorylated at Ser129 in dementia with Lewy bodies and Parkinson's disease. Alzheimers Res. Ther. 6, 77. https://doi.org/10.1186/s13195-014-0077-y.

Toba, S., Jin, M., Yamada, M., Kumamoto, K., Matsumoto, S., Yasunaga, T., Fukunaga, Y., Miyazawa, A., Fujita, S., Itoh, K., Fushiki, S., Kojima, H., Wanibuchi, H., Arai, Y., Nagai, T., Hirotsune, S., 2017. Alpha-synuclein facilitates to form short unconventional microtubules that have a unique function in the axonal transport. Sci. Rep. 7, 1–19. https://doi.org/10.1038/s41598-017-15575-3.

Torres-Garcia, L., Domingues, P., Brandi, E., Haikal, C., Mudannayake, J.M., Brás, I.C., Gerhardt, E., Li, W., Svanbergsson, A., Outeiro, T.F., Gouras, G.K., Li, J.-Y., 2022. Monitoring the interactions between alpha-synuclein and tau in vitro and in vivo using bimolecular fluorescence complementation. Sci. Rep. 12, 2987. https://doi.org/10.1038/s41598-022-06846-9.

Tsai, R.M., Miller, Z., Koestler, M., Rojas, J.C., Ljubenkov, P.A., Rosen, H.J., Rabinovici, G.D., Fagan, A.M., Cobigo, Y., Brown, J.A., Jung, J.I., Hare, E., Geldmacher, D.S., Natelson-Love, M., McKinley, E.C., Luong, P.N., Chuu, E.L., Powers, R., Mumford, P., Wolf, A., Wang, P., Shamloo, M., Miller, B.L., Roberson, E. D., Boxer, A.L., 2020. Reactions to multiple ascending doses of the microtubule stabilizer TPI-287 in patients with Alzheimer disease, progressive Supranuclear palsy, and Corticobasal syndrome: A randomized clinical trial. JAMA Neurol 77, 215–224. https://doi.org/10.1001/jamaneurol.2019.3812.

Twohig, D., Nielsen, H.M., 2019. α-Synuclein in the pathophysiology of Alzheimer's disease. Mol. Neurodegener. 14, 23. https://doi.org/10.1186/s13024-019-0320-x.

Ulmer, T.S., Bax, A., Cole, N.B., Nussbaum, R.L., 2005. Structure and dynamics of micelle-bound human α-Synuclein. J. Biol. Chem. 280, 9595–9603. https://doi.org/10.1074/jbc.M411805200.

Utton, M.A., Noble, W.J., Hill, J.E., Anderton, B.H., Hanger, D.P., 2005. Molecular motors implicated in the axonal transport of tau and α-synuclein. J. Cell Sci. 118, 4645–4654. https://doi.org/10.1242/jcs.02558.

Uversky, V.N., 2002. What does it mean to be natively unfolded? Eur. J. Biochem. 269, 2–12. https://doi.org/10.1046/j.0014-2956.2001.02649.x.

Vacchi, E., Kaelin-Lang, A., Melli, G., 2020. Tau and alpha synuclein synergistic effect in neurodegenerative diseases: when the periphery is the core. Int. J. Mol. Sci. 21, 1–32. https://doi.org/10.3390/ijms21145030.

Visanji, N.P., Mollenhauer, B., Beach, T.G., Adler, C.H., Coffey, C.S., Kopil, C.M., Dave, K. D., Foroud, T.M., Chahine, L., Jennings, D., 2017. The systemic Synuclein sampling study: toward a biomarker for Parkinson' s disease. Biomark. Med 11, 359–368.

Visanji, N.P., Lang, A.E., Kovacs, G.G., 2019. Beyond the synucleinopathies: alpha synuclein as a driving force in neurodegenerative comorbidities. Transl Neurodegener 8, 28. https://doi.org/10.1186/s40035-019-0172-x.

Waites, C., Qu, X., Bartolini, F., 2021. The synaptic life of microtubules. Curr. Opin. Neurobiol. 69, 113–123. https://doi.org/10.1016/j.conb.2021.03.004.

Wang, Y., Mandelkow, E., 2016. Tau in physiology and pathology. Nat. Rev. Neurosci. 17, 5–21. https://doi.org/10.1038/nrn.2015.1.

Wong, Y.C., Krainc, D., 2017. α-Synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies. Nat. Med. 23, 1–13. https://doi.org/10.1038/nm.4269.

Xueping, Yang, Huihan, Ma, Qingyun, Yv, Fanlong, Ye, Zhang, He, Sheng, Chen, Alimu, Keram, Wenwei, Li, Min, Zhu, 2022. Alpha-synuclein/MPP+ mediated

activation of NLRP3 inflammasome trough microtubule-driven mitochondrial perinuclear transport. Biochem. Biophys. Res. Commun. 594, 161–167. https://doi.org/10.1016/j.bbrc2022.01.047.

Yan, X., Uronen, R., Huttunen, H.J., 2018. The interaction of α-synuclein and tau: A molecular conspiracy in neurodegeneration? Semin. Cell Dev. Biol. 17, 1–10. https://doi.org/10.1016/j.semcdb.2018.05.005.

Yancopoulou, D., Xuereb, J.H., Crowther, R.A., Hodges, J.R., Spillantini, M.G., 2005. Tau and α-Synuclein inclusions in a case of familial frontotemporal dementia and progressive aphasia. J. Neuropathol. Exp. Neurol. 64, 245–253. https://doi.org/10.1093/jnen/64.3.245.

Zhang, X., Gao, F., Wang, D., Li, C., Fu, Y., He, W., Zhang, J., 2018. Tau pathology in Parkinson's disease. Front. Neurol. 9, 1–7. https://doi.org/10.3389/fneur.2018.00809.

Zhou, Y., Gu, G., Goodlett, D.R., Zhang, T., Pan, C., Montine, T.J., Montine, K.S., Aebersold, R.H., Zhang, J., 2004. Analysis of α-Synuclein-associated proteins by quantitative proteomics. J. Biol. Chem. 279, 39155–39164. https://doi.org/10.1074/jbc.M405456200.

Zhou, R.M., Huang, Y.X., Li, X.L., Chen, C., Shi, Q., Wang, G.R., Tian, C., Wang, Z.Y., Jing, Y.Y., Gao, C., Dong, X.P., 2010. Molecular interaction of α-synuclein with tubulin influences on the polymerization of microtubule in vitro and structure of microtubule in cells. Mol. Biol. Rep. 37, 3183–3192. https://doi.org/10.1007/s11033-009-9899-2.