Received: 10 July 2023 | Revised: 19 September 2023 | Accepted: 1 October 2023

DOI: 10.1111/jnc.15992

Journal of Neurochemistry JNC The Official Journal of the International Society for Neurochemistry WILEY

REVIEW

PrP meets alpha-synuclein: Molecular mechanisms and implications for disease

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Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: SFB1286 (B8) and EXC 2067/1-390729940; Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

Abstract

The discovery of prions has challenged dogmas and has revolutionized our understanding of protein-misfolding diseases. The concept of self-propagation via protein conformational changes, originally discovered for the prion protein (PrP), also applies to other proteins that exhibit similar behavior, such as alpha-synuclein (aSyn), a central player in Parkinson's disease and in other synucleinopathies. aSyn pathology appears to spread from one cell to another during disease progression, and involves the misfolding and aggregation of aSyn. How the transfer of aSyn between cells occurs is still being studied, but one important hypothesis involves receptor-mediated transport. Interestingly, recent studies indicate that the cellular prion protein (PrP^C) may play a crucial role in this process. PrP^C has been shown to act as a receptor/sensor for protein aggregates in different neurodegenerative disorders, including Alzheimer's disease and amyotrophic lateral sclerosis. Here, we provide a comprehensive overview of the current state of knowledge regarding the interaction between aSyn and PrP^C and discuss its role in synucleinopathies. We examine the properties of PrP and aSyn, including their structure, function, and aggregation. Additionally, we discuss the current understanding of PrP^C's role as a receptor/sensor for aSyn aggregates and identify remaining unanswered questions in this area of research. Ultimately, we posit that exploring the interaction between aSyn and PrP^C may offer potential treatment options for synucleinopathies.

Abbreviations: aSyn, alpha-synuclein; CJD, Creutzfeldt-Jakob disease; HSPG, heparan sulfate proteoglycans; Lag3, lymphocyte-activation gene 3; NKA, Na^+/K^+-ATPase; NMDAR, N-methyl-D-aspartate receptors; PD, Parkinson's disease; PrP, prion protein; TSEs, transmissible spongiform encephalopathies.

This article is part of the special issue "Protein aggregation in aging and neurodegeneration".

KEYWORDS
alpha-synuclein, neurodegeneration, Parkinson's disease, prion, prion diseases, PrP

1 | INTRODUCTION

1.1 | Prions and the expansion of the prion concept

The term "prion" refers to a protein responsible for causing various neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs), such as Creutzfeldt-Jakob in humans and mad cow disease in bovine cattle. Stanley Prusiner first coined the term in 1982, and the discovery of prions challenged the prevailing scientific understanding of infectious diseases, which had previously held that viruses, bacteria, or fungi were the only agents to cause infections (Prusiner, 1982).

Named after scrapie, the TSE affecting sheep, the infectious form of the prion protein (PrP^{Sc}) was hypothesized to be derived from a normal cellular protein called PrP^{C}. In 1986, researchers discovered the gene encoding the PrP^{C} protein. The gene was named *PRNP* and was found to be highly conserved across species (Basler et al., 1986). Over the years, it was also discovered that PrP^{C} was involved in various cellular processes, including cell adhesion, signal transduction, and neuroprotection (Legname, 2017; Wulf et al., 2017), but the functions of PrP^{C} are still not fully understood. Different mouse strains in which the PrP gene is ablated were generated to try to reveal its functions, but the phenotypes observed are still controversial (Marín-Moreno et al., 2020; Steele et al., 2007).

The capacity of PrP^{Sc} to act as an infectious agent depends on the presence of PrP^{C}, which gets converted into the PrP^{Sc} form (Prusiner et al., 1990). This self-propagating ability is connected to a change in protein conformation that leads to the formation of aggregates that act as a template that induces the conversion of normal cellular proteins into the misfolded conformation. This concept was later extended to several other proteins related to non-infectious diseases, such as other neurodegenerative diseases and cancer (Eraña et al., 2017; Scialò et al., 2019; Silva et al., 2013). For this, the terms "prionoid" or "prion-like" have been introduced and are now used for proteins that present a replicative cycle based on conformational remodeling. Since these other diseases seem to lack the infectious nature of TSEs, it is important to consider the use of terms that distinguish them to avoid confusion in the general public.

Identifying the prion-like behavior of proteins other than PrP suggests that the mechanisms of protein misfolding and aggregation that underlie prion diseases may be more widespread than previously thought. This also suggests that understanding the molecular underpinnings of protein aggregation and conversion may lead to the development of therapeutic strategies that may be more generally applicable (or adaptable) for slowing or halting the progression of various devastating disorders for which we still lack effective therapies.

1.2 | PrP structure, function, and aggregation

PrP^{C} is a glycoprotein found on the surface of cells in various organs and tissues, including the central nervous system (CNS) and peripheral nervous system (Bendheim et al., 1992). PrP^{C} is anchored to the cell membrane through glycosylphosphatidylinositol, primarily in lipid rafts. After it reaches the extracellular membrane, PrP^{C} can travel back to the cell interior for either recycling or degradation, through clathrin-dependent and caveolin pathways (Peters et al., 2003; Shyng et al., 1994).

PrP^{C} is composed of two structural domains: a flexible N-terminal domain and a globular C-terminal domain (Figure 1a; Donne et al., 1997; Riek et al., 1997). The N-terminal domain spans from amino acids 23 to 124 and consists of several distinct regions. These include two positively charged clusters known as CC1 and CC2, an octarepeat region (most mammalian PrP proteins contain five or six repeats of a peptide with 8 or 9 amino acid residues with the sequence P(Q/H)GGG(G/−)WGQ), and a hydrophobic domain (Figure 1a; Donne et al., 1997; Riek et al., 1997). The C-terminal domain consists of three α-helices and two antiparallel β-sheets, spanning from amino acids 125 to 230. It also has a disulfide bridge connecting cysteine 179 and cysteine 214. In addition, oligosaccharides can attach to asparagine 181 and asparagine 197 residues, resulting in mono-glycosylation or di-glycosylation (Haraguchi et al., 1989).

Interestingly, PrP mutations linked to familial forms of prion diseases are mostly located in the C-terminal domain, where they increase the propensity of the protein to aggregate (Eghiaian et al., 2004; Wopfner et al., 1999).

As stated earlier, the physiological functions of PrP^{C} are still elusive, but it seems to play a role in regulating sleep, as evidenced by studies in $PrP^{-/-}$ ZrchI mice which display disrupted circadian rhythms and more fragmented sleep (Tobler et al., 1996). Consistently, the interaction of PrP^{C} with monoaminergic receptors was considered essential for modulating melatonin synthesis and depressive-like behavior (Roguski & Gill, 2017). Another study, using a transgenic mouse model expressing the mouse homolog of the PrP variant linked to familial Creutzfeldt-Jakob disease (CJD; D178N/V129), reported anomalies in sleep and in electroencephalogram recordings, together with functional impairments in memory and motor abilities (Dossena et al., 2008). Sleep deprivation is associated with decreased levels of both PrP^{C} and mGluR1, and with an increase in the levels of Aβ peptides, suggesting that lack of sleep may have a detrimental impact on neuronal plasticity (da Luz et al., 2020). These data suggest that PrP^{C} interacts with various receptors and molecules that modulate sleep, mood, memory, and neuronal plasticity and that alterations in PrP^{C} expression or function may lead to sleep disturbances and cognitive impairments.

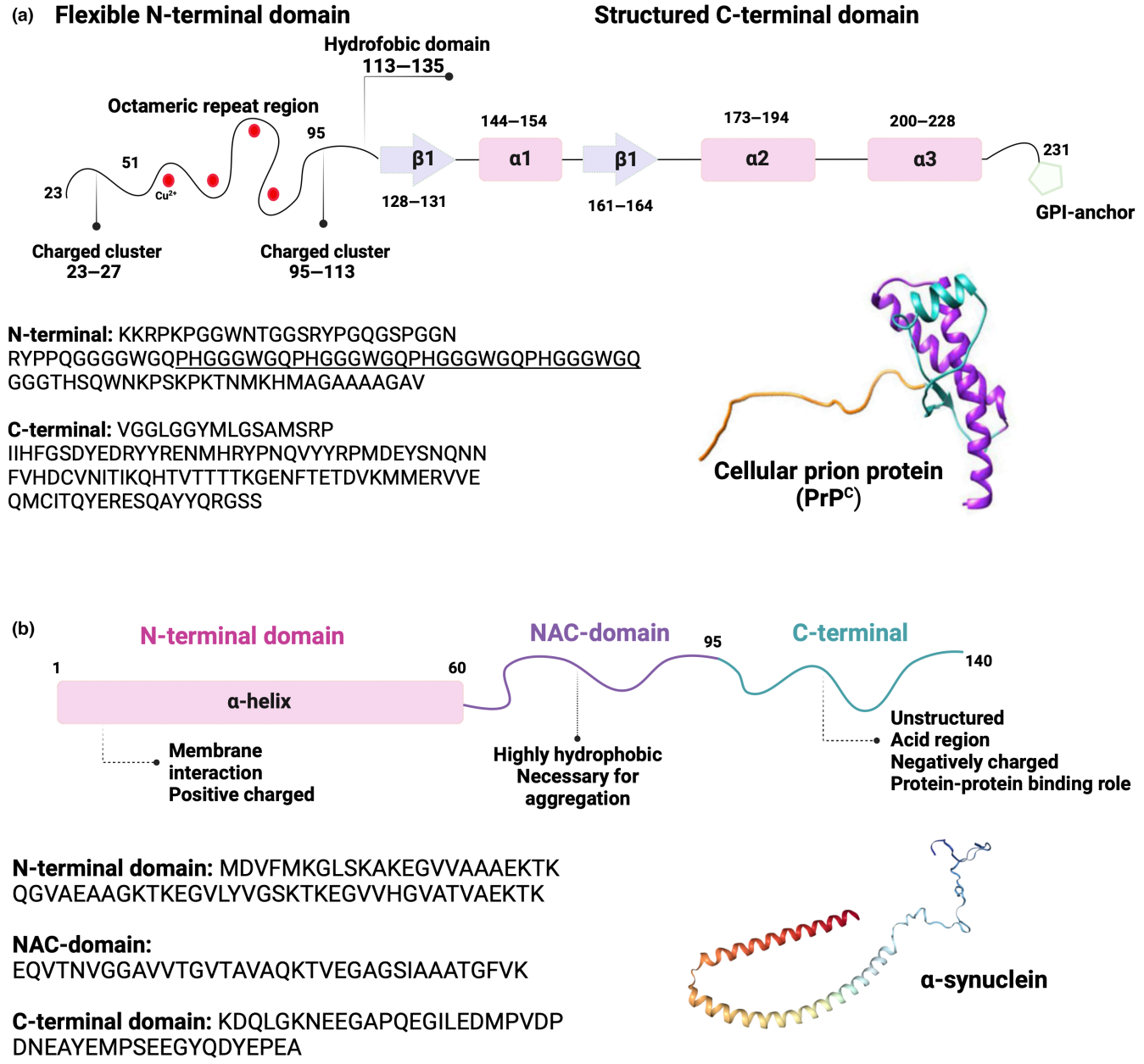


FIGURE 1 Amino acid sequence, domains, and structure of cellular prion protein (PrP^C) and alpha-synuclein (aSyn). (a) The mature PrP^C at the cell surface has a sequence of 209 amino acids (UNIPROT code P04156 depicted) divided into N- and C-terminal domains. The unstructured N-terminal domain has charged and hydrophobic amino acid clusters and an octapeptide repeat region (underlined) that interacts with different molecules. The globular C-terminal domain comprises three α-helices and a small antiparallel β-sheet. (b) aSyn (UNIPROT code P37840 depicted) is an intrinsically disordered protein composed of 140 amino acids, and its structure is divided into three domains: the N-terminal, non-amyloid component (NAC), and C-terminal domain. The N-terminal region is positively charged and gains an α-helix coiled structure after interaction with lipid membranes. This α-helix coiled structure extends to the NAC domain. The NAC domain is highly hydrophobic and involved in fiber formation, and the C-terminal is negatively charged and shows a random coil structure. Created with BioRender.com.

Additional research has demonstrated that PrP^C plays a significant role in supporting neuronal growth during developmental stages and in adult neurogenesis (Brown et al., 1997; Dupiereux et al., 2008; Kim et al., 2004; Nuvolone et al., 2013; Rangel et al., 2009; Steele et al., 2006; Weise et al., 2004). In fact, the neuroprotective function of PrP^C was connected to its interaction with copper ions and N-methyl-D-aspartate receptors (NMDAR), regulating NMDAR nitrosylation and activity by reducing the influx of calcium ions into neurons (Gasperini et al., 2015; Stys et al., 2012). Strikingly, calcium ion dysregulation is a critical factor in various neurodegenerative processes, so the regulation of this process by PrP^C can be seen as neuroprotective (Zündorf & Reiser, 2011).

The binding of PrP^C to neural cell adhesion molecule (NCAM) promotes cell adhesion, particularly in neurons, contributing to the

establishment and maintenance of neuronal networks (Santuccione et al., 2005; Schmitt-Ulms et al., 2001; Slapšak et al., 2016). NCAM is a transmembrane glycoprotein involved in cell adhesion and signal transduction in the nervous system, playing a crucial role in neural development, synaptic plasticity, and neuronal migration (Ditlevsen et al., 2008). The interaction with PrP^C enhances NCAM-mediated signaling pathways, including activating intracellular kinases such as Fyn, which are important regulators of synaptic plasticity (Santuccione et al., 2005).

The interaction between PrP and its various partners is not only crucial for its many physiological functions but also it plays a role in the development of prion diseases. In prion diseases, PrP is converted from the normal cellular form (PrP^C) into the misfolded, aggregated form PrP^{Sc}, which can then propagate and spread throughout the body. This conversion is thought to occur through a templating mechanism, in which PrP^{Sc} acts as a template that induces the misfolding of PrP^C (Bueler et al., 1993).

Alongside with the templating mechanism, the pathogenicity of PrP is influenced by its interaction with other partners, such as nucleic acids, lipids, and polysaccharides, all of which can enhance its propagation and spread (Alves Conceição et al., 2022; Silva et al., 2022; Supattapone, 2014).

Thus, the ability of PrP to interact with multiple partners constitutes a "double-edged sword." While these interactions are important for its normal physiological functions, they can also contribute to its pathogenic role in prion diseases. A greater understanding of these interactions is necessary to enable the development of therapeutic strategies for treating these devastating diseases.

1.3 | PrP^C: A troublesome receptor at the cell surface

Recent studies have revealed that PrP^C may act as a receptor/sensor for different protein aggregates, modulating different neuropathologies both in terms of neuronal toxicity and transmission of pathogenic aggregates to adjacent regions (Resenberger et al., 2011; Scialò & Legname, 2020). This discovery has important implications for understanding the development of neurodegenerative disorders and provides novel possibilities for treatment.

The interaction between PrP^C and amyloid-β (Aβ) peptides, which are central to the pathology of Alzheimer's disease (AD), is an example of how PrP^C may act as a troublesome receptor. Although PrP^C is not the only receptor for Aβ at the cell surface (Benilova et al., 2012; De Strooper & Karran, 2016), the interaction between PrP^C and Aβ enhances the toxicity of Aβ, exacerbating neuronal damage and promoting the formation of amyloid plaques. This interaction appears oligomeric-specific and occurs via the CC region of PrP^C (amino acids 23–27 and 94–110; Freir et al., 2011). The mechanism of toxicity seems to involve Fyn kinase, a member of the Src family of kinases (SFKs), that has been associated with diverse functions in the CNS including myelination and synaptic transmission. Dysfunction of Fyn kinase is associated with neurodegenerative diseases (Guglietti et al., 2021). Fyn kinase phosphorylates NMDAR, increasing calcium influx, and initiating long-term potentiation (LTP; Trepanier et al., 2012). However, sustained NMDAR phosphorylation leads to excitotoxicity (Um et al., 2012). The Aβ oligomer-PrP^C interaction leads to phosphorylation of the Fyn kinase via the metabotropic glutamate receptor 5 (mGluR5) and subsequent phosphorylation of the NMDAR2B, leading to synaptic dysfunction in the hippocampus, LTP deficits, and destabilization of the dendritic spines. This phosphorylation cascade has also been associated with increased hyperphosphorylated Tau levels, affecting the progression of AD (Salazar & Strittmatter, 2017; Um et al., 2012).

Recent research revealed that AD brains contain different types and levels of PrP^C isoforms (reviewed in Zhang et al., 2019). This profile changes with the progression and severity of the disease, providing a potential tool for diagnosing and evaluating AD. Additionally, PrP^C has been detected in amyloid plaques in AD (Schwarze-Eicker et al., 2005; Takahashi et al., 2011).

The function of PrP^C in AD is still unclear, but reduced PrP^C expression in the hippocampus is linked to aging and an increased risk of AD, suggesting a protective role in pathology (Whitehouse et al., 2010). Some studies focused on measuring PrP^C levels in different AD Braak stages and found that the levels increase in the initial stages of the pathology but decrease in later stages, leading to neuronal death (Vergara et al., 2015). Differences in glycosylated forms of PrP^C have also been reported (Saijo et al., 2011). Nevertheless, other studies found no significant differences in PrP^C expression between healthy individuals and those with AD (Abu Rumeileh et al., 2017; Dohler et al., 2014).

PrP^C was shown to also interact with Tau aggregates and promote their internalization, thereby facilitating the propagation of tau pathology (Corbett et al., 2020; De Cecco et al., 2020). Likewise, PrP^C was shown to interact with TDP-43, a protein that affects neurons in amyotrophic lateral sclerosis and in frontotemporal lobar degeneration, increasing the uptake of TDP-43 fibrils and modulating toxicity (Scialò et al., 2021). PrP^C has also been implicated in the pathogenesis of Parkinson's disease (PD) as it was shown to interact with alpha-synuclein (aSyn), a major component of Lewy body inclusions in the brains of PD patients, leading to increased cellular toxicity and the spread of pathological aggregates (Ferreira et al., 2017).

The interaction between PrP^C and various protein aggregates or with aggregation-prone proteins adds a layer of complexity to prion disease pathogenesis and may influence the amplification and spread of prion pathology.

1.4 | a-Synuclein structure, function, and aggregation

aSyn is an intracellular 140 amino acid protein abundantly expressed in neurons (Murphy et al., 2000). In its soluble state, aSyn is an intrinsically disordered protein, but it can acquire structured segments when bound to membranes (Eliezer et al., 2001; Park et al., 2002). This structural flexibility may explain its multiple interacting

partners and the variety of functions it has been associated with (Brás et al., 2020).

aSyn can be divided in three distinct domains according to its primary sequence: (i) the N-terminal domain (1–60) contains a multi-repeated imperfect consensus hexameric sequence (KTKEGV) that make it amphipathic and positively charged, adopting alpha-helical conformation when interacting with membranes (Burré et al., 2010; Davidson et al., 1998; Jo et al., 2000); (ii) the Non-Amyloid Component Domain (61–95) is a central hydrophobic region, essential for aSyn aggregation, and contains additional KTKEGV repeats; (iii) the C-terminal domain (96–140) contains a stretch of negatively charged residues enabling the protein to interact with various small molecules and proteins such as vesicle-associated membrane protein 2 and Synaptobrevin-2, two regulators of synaptic exocytosis and endocytosis, respectively (Figure 1b; Burré et al., 2010; Sun et al., 2019).

Experiments using aSyn knock-out (KO)/knock-down, or overexpression have been conducted to gain insight into its physiological function. Most notably, aSyn plays a regulatory role in neurotransmitter release, synaptic function, and plasticity (Abeliovich et al., 2000; Brás et al., 2020; Burré, 2015; Burré et al., 2010; Domingues et al., 2022; Marques & Outeiro, 2012). However, aSyn can also be detected in the nucleus, where it is assumed to be involved in transcriptional regulation and regulation of histone acetylation (Goers et al., 2003; Kontopoulos et al., 2006; Koss et al., 2022; Pinho et al., 2019). In the presence of lipid vesicles, aSyn is found in a folded, membrane-bound state (Liu et al., 2021), where the N-terminal region connects with the central region, maintaining the C-terminal unstructured (Jao et al., 2008; Runfola et al., 2020).

aSyn-KO mice do not display major phenotypes or abnormalities but, upon closer examination, alterations in synaptic vesicle modifications, decreased striatal dopamine and neurotransmission acceleration, and inhibition of the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) complex assembly have been observed (Burré et al., 2010; Butler et al., 2017). In addition, neurons depleted of aSyn have impaired synaptic vesicle mobilization (Cabin et al., 2002), and reduced synaptic vesicle pool reserves (Murphy et al., 2000).

On the other hand, overexpression of aSyn in mice and in primary neurons decreases vesicle release and recycling after endocytosis, leading to mislocalization of SNARE proteins and sequestration of aSyn aggregates on SNARE proteins, such as synaptobrevin-2 causing a blockade on vesicle docking (Choi et al., 2013; Garcia-Reitböck et al., 2010; Nemani et al., 2010). An excess of aSyn also reduces dopamine reuptake, via dopamine active transporter, and dopamine production via tyrosine hydroxylase, causing impairment in neurotransmitter transport (Hansen et al., 2013; Masliah et al., 2000). In animal models, overexpression of aSyn results in redistribution of synaptic vesicles to locations further from the active zone and, in hippocampal neurons, it can induce the loss of synaptic proteins and enlarged vacant vesicles, leading to abnormalities in vesicle priming, fusion, and docking (Janezic et al., 2013).

aSyn aggregation into insoluble structures known as Lewy bodies and Lewy neurites is a pathognomonic pathological hallmark of PD. While the role of aSyn aggregates is still controversial, the prevailing theory is that some types of aSyn-containing aggregates might be neurotoxic, contributing to neuronal dysfunction and, eventually, death.

Synucleinopathies are a group of neurodegenerative diseases characterized by the progressive accumulation of aSyn-containing protein aggregates in different cells of the nervous tissue, such as neuronal and glial cells. Synucleinopathies include PD, multiple system atrophy, and dementia with Lewy bodies (Brás et al., 2020). Recent studies using cryo-electron microscopy suggest that the arrangement of aSyn in fibrillar material extracted from brain tissue from individuals who had a synucleinopathy may be different (Guerrero-Ferreira et al., 2018, 2019; Li, Ge, et al., 2018; Li, Zhao, et al. 2018), but additional studies on this topic are still necessary.

During the early stages of fibrillization, aSyn undergoes partial folding into a pre-molten globule-like conformation (Uversky et al., 2001). The precise molecular factors involved in this conformational change are still unclear, but likely include protein concentration imbalances, mutations, posttranslational modifications (PTMs), and environmental factors such as changes in pH, salt concentration, inflammation, or the presence of chemical factors such as polyamines (Antony et al., 2003; Fujiwara et al., 2019; Guzzo et al., 2021; Manzanza et al., 2021; Sandal et al., 2008; Wu et al., 2009). The oligomerization process likely begins with the formation of dimers, followed by the formation of soluble and non-fibrillar oligomeric species with different morphologies, such as annular, chain-like, or spherical structures. Upon reaching a critical concentration, some of these oligomers may convert into protofilaments, protofibrils, and other high-molecular-weight species that can be amorphous or amyloid-like aggregates (Figure 2; Hijaz & Volpicelli-Daley, 2020).

Under certain conditions, spherical oligomers may convert into ring-like structures, which can permeabilize membranes in in vitro studies, by forming pore-like structures that affect membrane potential and ion distribution (Kim et al., 2009; Lashuel et al., 2002). However, such pore-forming structures have never been observed in biological systems (cells or brain tissue). Current evidence suggests that aSyn oligomers and protofibrils, rather than larger insoluble aggregates, may be the most cytotoxic species (Cascella et al., 2021; Outeiro et al., 2008; Tanaka et al., 2004; Winner et al., 2011). Consistently, several studies aimed at stabilizing aSyn oligomers report increased cytotoxicity (Fusco et al., 2017; Ingelsson, 2016), but this is also a matter of intense debate in the field.

1.5 | Synucleinopathies as prion-like diseases

The analogy between prion diseases and synucleinopathies is mainly because of the apparent spreading of protein pathology from cell to cell, region to region, and even organ to organ (Holmqvist et al., 2014). Braak et al. (2003) hypothesized that the topography of synucleinopathies is related to the severity of the clinical symptoms, and that disease progression could be separated into distinct stages. In PD, the

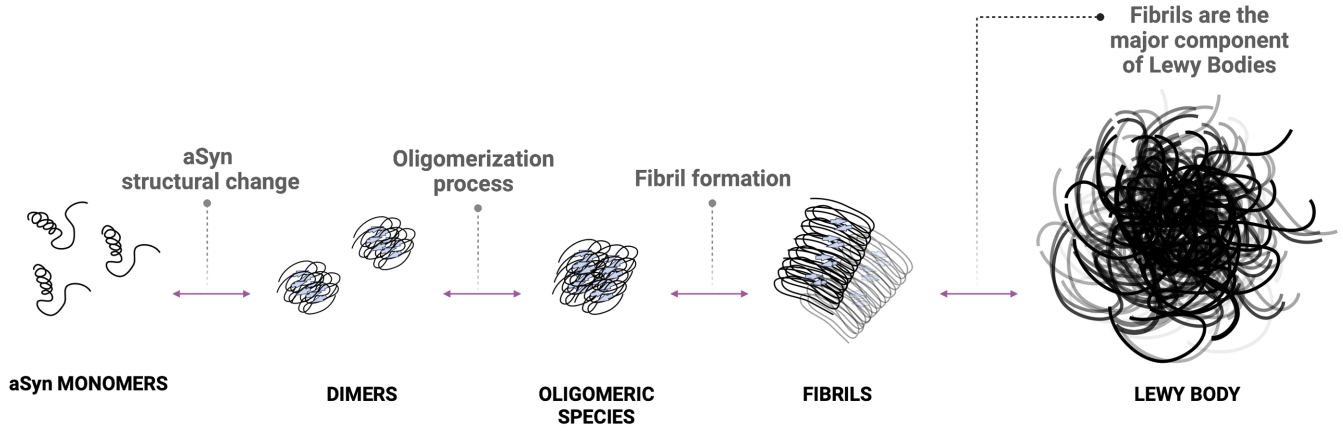


FIGURE 2 Schematic representation of alpha-synuclein (aSyn) in Lewy body formation. Monomeric aSyn undergoes structural changes that increase the probability of forming dimers, oligomers, and other aggregated species. Once formed, these aggregated species can further form amyloid fibers that are the major component of Lewy bodies. Created with BioRender.com.

first stage is characterized by olfactory deficits, constipation, and sleep disturbances, which reflect the involvement of the olfactory bulb, enteric system, and caudal brainstem, respectively. In the subsequent stages, motor symptoms may correspond to a time when aSyn pathology has reached the substantia nigra. At the final stages, the pathology reaches the neocortex, resulting in psychiatric symptoms and cognitive decline (Braak et al., 2003; Fullard et al., 2017).

The exact mechanisms underlying the transfer of aSyn aggregates are still being actively investigated. These aggregates can then be released and taken up by neighboring cells through various processes that include, but are not necessarily limited to, direct diffusion of aSyn through the cellular membrane, endoplasmic reticulum-Golgi-dependent exocytosis, aSyn secretion in extracellular vesicles, tunneling nanotubes, or receptor-mediated endocytosis (Figure 3; Danzer et al., 2012; Emmanouilidou et al., 2010).

Once internalized by recipient cells, aSyn aggregates may induce the misfolding and aggregation of intracellular aSyn, thus perpetuating the cycle of aggregate formation (Masuda-Suzukake et al., 2013; Paumier et al., 2015). Experiments using aSyn KO mice showed that endogenous aSyn is essential for the spreading of pathology, a process that does not require aSyn toxicity (Dening et al., 2022). Consequently, transmitting aSyn aggregates between cells may contribute to the progression of neurodegeneration observed in PD and related disorders.

Interestingly, experiments using proteinase K aimed at decreasing the abundance of cell surface proteins have revealed a correlation between reduced levels of aSyn internalization and protease treatment, suggesting cell surface proteins contribute to the spreading of pathology (Lee et al., 2008).

One of these cell-surface proteins is lymphocyte-activation gene 3 (LAG3), an immunoglobulin family member expressed in neurons, microglia, and immune cells (Anderson et al., 2016; Liu et al., 2018). LAG3 can bind to recombinantly produced mouse aSyn pre-formed fibrils (PFFs) via its D1 domain, colocalizing with multiple Rab proteins and endosomal GTPases implicated in the internalization of extracellular aSyn (Mao et al., 2016; Figure 4a). However, the involvement of LAG3 in the spreading of aSyn pathology is controversial, as some studies presented conflicting findings regarding the levels of expression of LAG-3, which seem to be negligible in both mouse and human neurons (Emmenegger et al., 2021). Nevertheless, while it remains possible that an interaction between certain aSyn aggregated species and LAG-3 may occur in disease settings, this does not appear to be the only relevant receptor to be considered.

The Na^+/K^+-ATPase (NKA) is a membrane protein that utilizes ATP to import two potassium ions and export three sodium ions. NKA is composed of three subunits, and the α3 subunit is linked to several neurodegenerative diseases (Geering, 2008; Ohnishi et al., 2015; Petrushanko et al., 2016; Ruegsegger et al., 2016; Shrivastava et al., 2015), and plays a crucial role in diverse cellular processes, including neuronal activity (Clausen et al., 2017). The α3 subunit interacts with Aβ, superoxide dismutase 1, and aSyn, and is hypothesized to regulate the endocytosis of these proteins into neuronal cells (Ohnishi et al., 2015; Ruegsegger et al., 2016; Shrivastava et al., 2015). This interaction leads to the entrapment of α3-NKA within clusters of aSyn, resulting in a decline in the efficiency of sodium export following an action potential and dysregulation of the neuronal refractory period (Figure 4b). α3-NKA was identified, along with neurexin 1α and 2α, using proteomic analyses, highlighting its potential involvement in synapse function (Shrivastava et al., 2015).

1.6 | Interactions between aSyn and PrP

PrP and aSyn have been found to interact and influence each other's behavior (De Cecco & Legname, 2018). In particular, PrP^C has been shown to regulate the internalization of aSyn and to act as a mediator of neuronal damage caused by aSyn oligomers (Aulić et al., 2017; Corbett et al., 2020; Ferreira et al., 2017). Interestingly, PrP^{Sc} infection increases aSyn phosphorylation at serine 129, a PTM considered a marker of LB pathology (Chen et al., 2021).

Soluble aSyn aggregates were suggested to interact with PrP at the cell surface (Figure 5a), and this was connected to toxicity and to

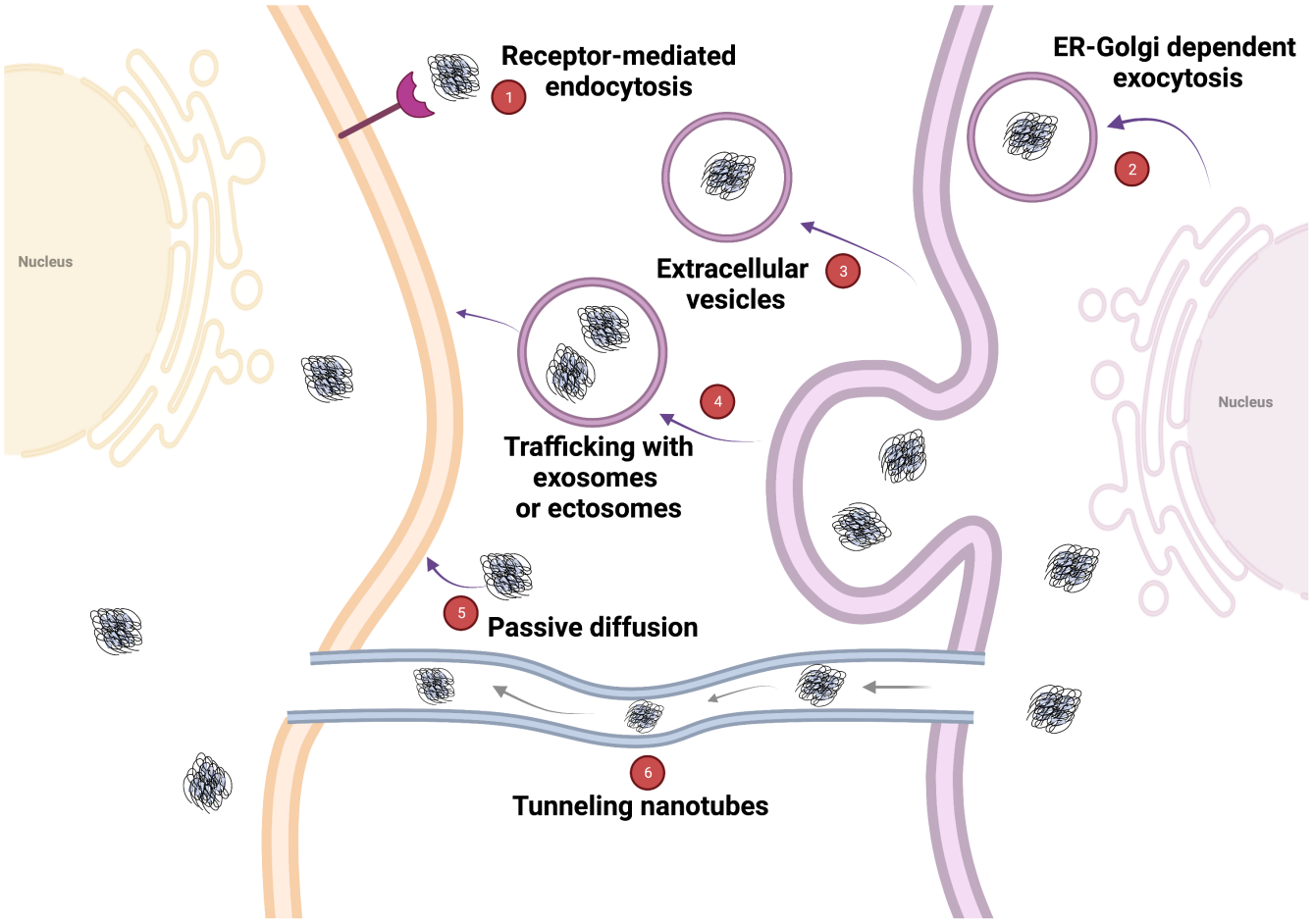


FIGURE 3 Possible mechanisms of alpha-synuclein (aSyn) spread between cells. aSyn transmission between two cells is hypothesized to occur through several mechanisms. (1) Receptor-mediated endocytosis may transport aSyn aggregates into the cell. Other possible mechanisms include (2) Golgi-mediated exocytosis and (3 and 4) extracellular vesicles, exosomes, and ectosomes that can fuse with the membrane of another cell. Aggregates can be internalized into neighboring cells (5) by passive diffusion and (6) move through tunneling nanotubes. Created with BioRender.com.

impairment of synaptic plasticity (Corbett et al., 2020), but the role of PrP^C in the pathology of PD, remains uncertain.

Based on existing literature, the interaction between PrP and aSyn is likely dependent on the type of aSyn species (monomeric, oligomeric, fibrillar, etc.).

Using solid-phase assays, fibrillar and monomeric aSyn showed no or weak affinity to full-length PrP^C (PrP 23–231; Corbett et al., 2020). In contrast, soluble aSyn aggregates are bound with high affinity to PrP 23–231 (in the nanomolar range; Corbett et al., 2020). However, it is important to recognize that these differences may also be as a result of the nature of the assay, since aggregated proteins tend to be "sticky," exhibiting a greater propensity to adhere and display binding in assays performed on solid surfaces when compared to soluble species (Corbett et al., 2020). While a study using surface plasmon resonance reported no interaction between PrP^C and aSyn oligomers (La Vitola et al., 2019), another demonstrated the interaction between full-length PrP and aSyn oligomers and monomers, although with higher affinity for oligomers (Thom et al., 2021).

In these solid-phase assays, the interaction with soluble aSyn aggregates occurred mainly through the N-terminus of PrP since the affinity was 8-times weaker with PrP 91–231, and no binding was detected with PrP 119–231 (Corbett et al., 2020). These data suggest that the binding site of these aSyn species resides mainly between residues 23 and 90 of PrP. However, using a more physiological assay, we have previously reported that PrP^C residues from 93 to 109 are involved in the interaction of aSyn oligomeric species with PrP in rat hippocampal brain slices, as we could block the effects of aSyn using the PrP antibody 6D11 (targeting the epitope region 93–109 of PrP^C; Ferreira et al., 2017).

It was also shown that monomeric aSyn and PrP interact in vitro, leading to the formation of liquid droplets that are highly dynamic, thermo-responsive, and reversible through the interaction of the N-terminal region of PrP^C with the C-terminal region of aSyn. In addition, aSyn-PrP condensates appear more prone to transitioning to a solid fibrillar state (Agarwal et al., 2022).

The controversy in the findings described here suggests that differences in the experimental systems need to be further dissected,

by conducting systematic studies using different methods and different aSyn species.

1.7 | The role of PrP on aSyn toxicity

As reported for Aβ oligomers, the connection between PrP^C and NMDAR can be unbalanced by aSyn aggregates. The interaction of PrP^C with oligomeric aSyn leads to Fyn kinase-mediated phosphorylation of mGluR5, activating the NMDAR2B and, ultimately, leading to an increase in intracellular calcium that causes synaptic dysfunction, including LTP impairment (Figure 5c; Ferreira et al., 2017). In addition, this interaction has been hypothesized to promote the formation of cofilin/actin rods by rearranging the cytoskeleton and affecting actin dynamics, blocking axonal transport (Brás et al., 2018; Silva et al., 2021).

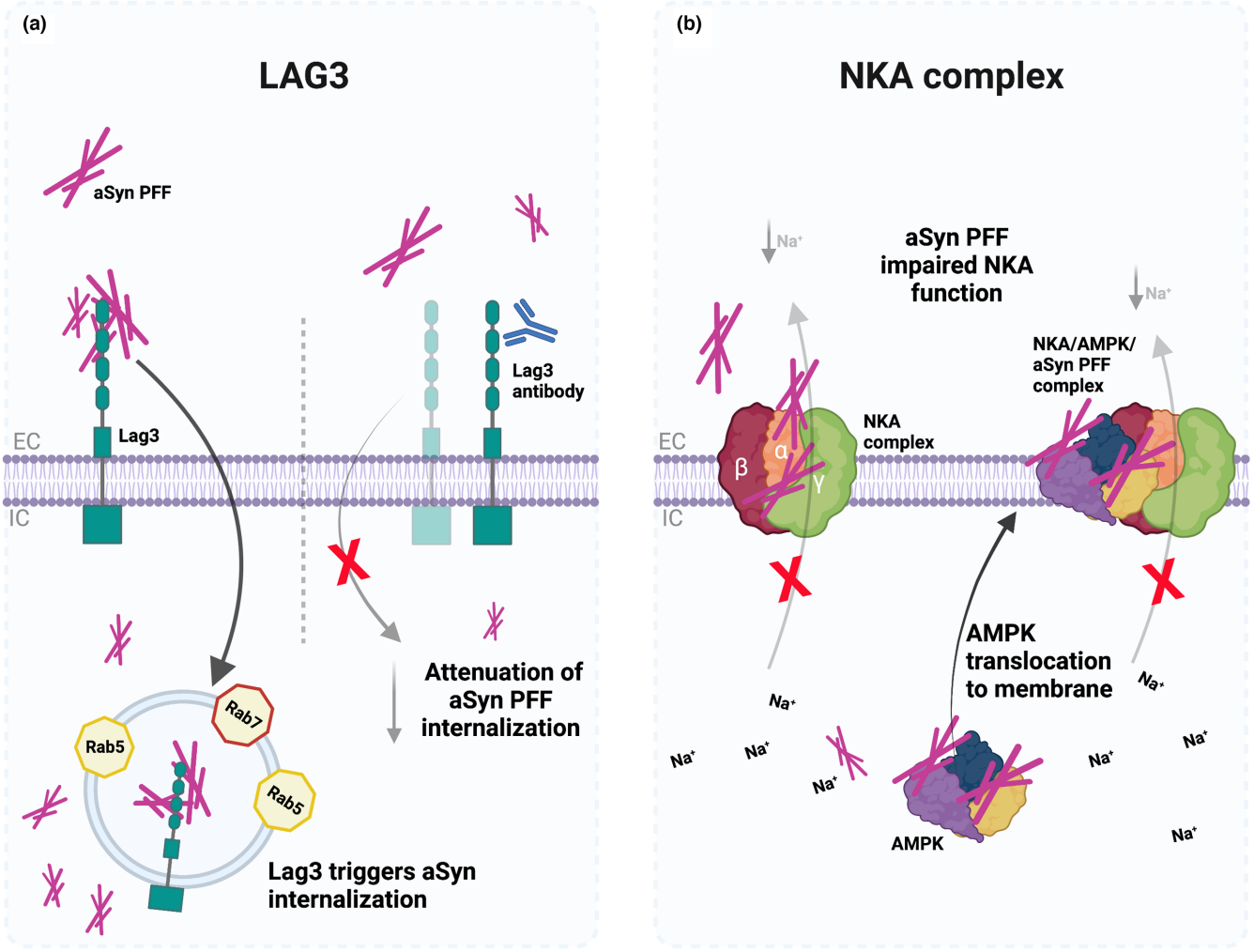
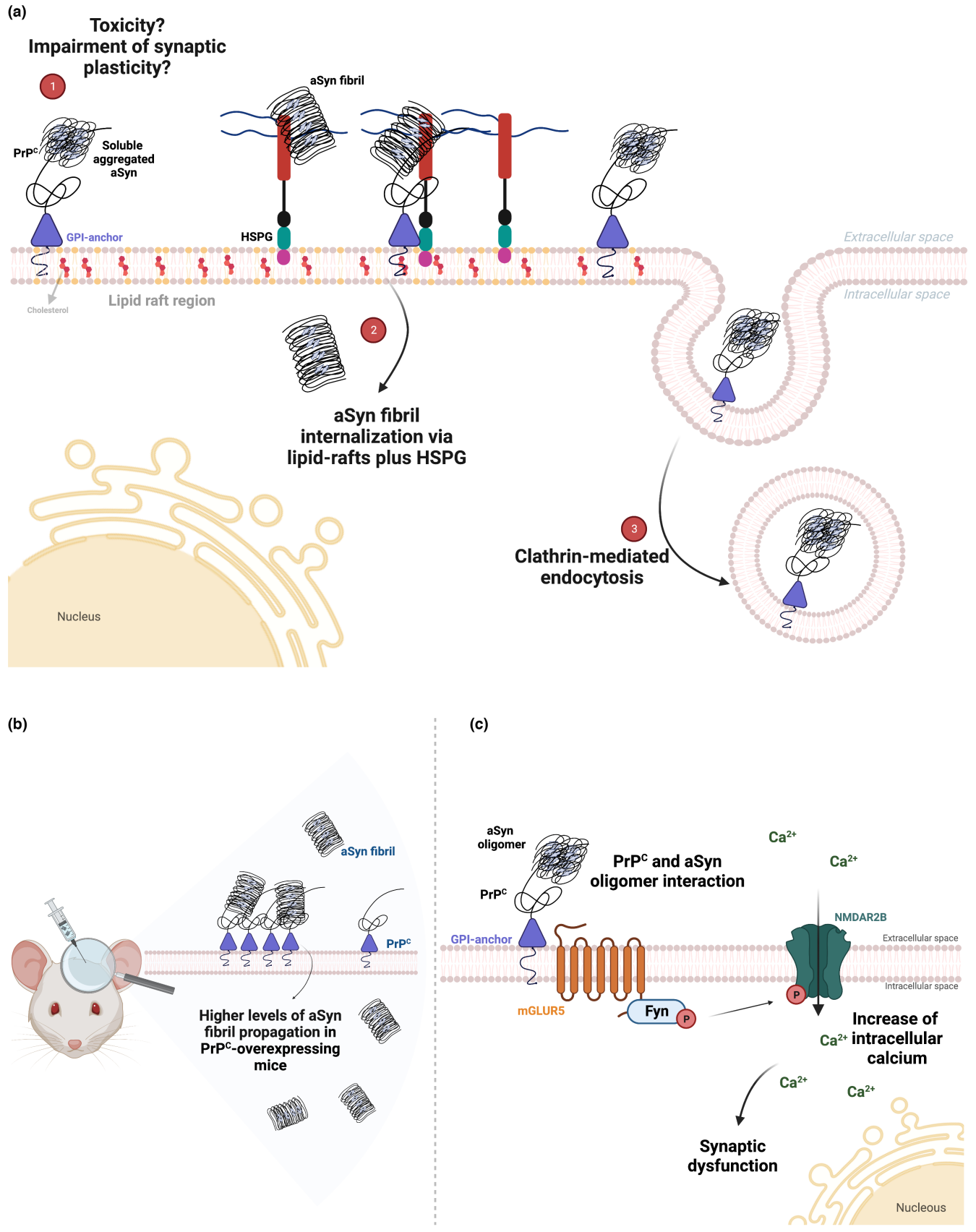


FIGURE 4 Lymphocyte-activation gene 3 (Lag3) and Na^+/K^+-ATPase (NKA) are involved in the internalization of alpha-synuclein (aSyn) preformed fibers (PFF). (a) Lag3 can directly interact with aSyn PFF, promoting their internalization across the cell membrane. Rab5 and Rab7 participate in this process. The absence of Lag3, or the use of Lag3 antibodies, attenuates the propagation of aSyn PFF. (b) The presence of aSyn aggregates impairs NKA function by interacting with the extracellular segments of α3-NKA. Another possibility is that the interaction of aSyn PFF with AMPK may contribute to the translocation of AMPK to the cell membrane and promote interaction with NKA. Thus, NKA function may also be impaired by the formation of the NKA/AMPK/PFF complex. Created with BioRender.com.

FIGURE 5 Cellular prion protein (PrP^C)-mediated internalization of alpha-synuclein (aSyn). (a) (1) The interaction between PrP^C and aggregated aSyn may contribute to toxicity and impairment of synaptic plasticity. (2) Heparan sulfate proteoglycans (HSPG) are involved with internalizing aSyn and PrP^{Sc} aggregates. One hypothesis is that the presence of PrP^C in HSPG-rich regions is involved with the internalization of aSyn fibrils. (3) The internalization of PrP-aSyn aggregates must take place via clathrin-mediated endocytosis. (b) Although PrP^C is not exclusive for spreading aSyn aggregates, its overexpression increases fibril spreading in mouse and cell models. (c) One mechanism for PrP^C-mediated cytotoxicity is the activation of NMDAR2B receptors. Interaction of PrP^C with oligomeric aSyn promotes the formation of PrP^C–mGluR5–Fyn cluster, activating NMDAR2B and promoting changes in intracellular calcium homeostasis. Dysregulation of calcium levels in cells is critical for neurodegeneration and cell death. Created with BioRender.com.



As mentioned earlier, PrP^C is a known partner of NKAs, thereby promoting ion uptake (Williams et al., 2021). Consistently, NKA inhibition by cardiac glycosides reduces both NKA and PrP^C levels (Mehrabian et al., 2022) suggesting the interdependence of these molecules. Since interfering with NKA activity can lead to calcium signaling dysregulation and, ultimately, to neuronal death (Kinoshita

et al., 2022), it is possible that the interaction between aSyn and PrP^C may disturb the interaction of NKAs with PrP^C, thereby exacerbating aSyn toxicity. Thus, we consider that modulating these interactions should be further investigated as a possible strategy for therapeutic intervention.

Nevertheless, contrasting results were also reported, questioning the importance of PrP^C for aSyn toxicity. In this case, primary hippocampal neurons from PrP WT or from PrP KO mice ($PrP^{-/-}$ ZrchI) were shown to be equally susceptible to aSyn oligomers, as both mice had equal memory impairment and neuroinflammatory responses, suggesting that aSyn toxicity was independent of PrP^C (La Vitola et al., 2019). However, it is not possible to rule out that the role of PrP^C as a receptor/sensor for aSyn may vary depending on the type of aSyn species, the brain region, and other as-of-yet unidentified factors.

1.8 | The role of PrP on the spreading of aSyn pathology

The precise mechanism(s) involved in the spreading of aSyn pathology is/are still elusive. aSyn can transfer between cells via passive and active mechanisms. Endocytic pathways are responsible for the internalization of most aSyn, especially aggregated aSyn (Neupane et al., 2023). The receptor-mediated internalization through PrP^C is an active mechanism and is clathrin-mediated (Thom et al., 2021; Figure 5a).

PrP^C interacts with heparan sulfate proteoglycans (HSPG), another co-receptor for various protein aggregates at the cell surface, and HSPG facilitates PrP^{Sc} propagation (Vieira & Silva, 2016). Since HSPG is essential for mediating the internalization of aSyn fibrils via a lipid-raft-dependent route (Hudák et al., 2019), the presence of PrP^C in HSPG-rich regions may facilitate aSyn internalization (Figure 5a). However, the extent to which the interaction between these partners can mediate and modulate aSyn internalization still needs further investigation.

While deletion of *PRNP* in mice ($PrP^{-/-}$ ZrchI) does block the spreading of aSyn, overexpression of PrP^C (Tga20) enhances the spreading of aSyn pathology in a model using stereotactic injections of aSyn PFFs (Urrea et al., 2018; Figure 5a, b).

In primary cortical neurons (from WT and $PrP^{-/-}$ ZrchI mice) and in SH-SY5Y cells (WT and overexpressing PrP^C using the pCI-neoPRNP vector), the PrP^C was found to increase the uptake of aSyn monomers and oligomers without triggering cytotoxicity. Nevertheless, the same study showed that PrP^C plays a role in the behavioral alterations and in the shorter lifespans observed in mice with aSyn pathology (Thy1-Syn, a transgenic line that displays a more rapidly progressive α-synucleinopathy phenotype; Thom et al., 2021). Ablation of *PRNP* in Thy1-Syn PrP0/0 mice (a cross between Thy1-Syn and $PrP^{-/-}$ ZrchI mice) ameliorates the behavioral deficits in Thy1-Syn mice (Thom et al., 2021). These data reinforce the hypothesis that PrP^C contributes to aSyn internalization and disease progression, possibly by acting as a receptor at the cell surface.

1.9 | Outlook: Implications for neurodegeneration

The discovery of cellular prion protein as a receptor (or sensor) for protein aggregates has provided valuable insight into the mechanisms underlying neurodegenerative diseases, including synucleinopathies. Nevertheless, the relationship between PrP and aSyn is likely complex and may not be equally relevant for different synucleinopathies, since different aSyn fibrillar species seem to occur in different synucleinopathies, as mentioned earlier. Additionally, since different pools of aSyn species may be present at different stages of disease, the relevance of the interaction may also vary during disease progression. In earlier stages, when soluble oligomeric species may be dominant, the interaction may lead to neuronal dysfunction, whereas at later stages it may be more relevant for the spreading of pathology. All of this will need to be considered for the design of effective therapeutic strategies.

An additional issue is that PrP is probably not the only player involved in the uptake and spreading of aSyn. To solve this, we must develop more specific models where the contribution of different pathways can be isolated and then introduced in a stepwise manner in order to establish causality/synergistic effects.

Nevertheless, the possible contribution of the aSyn-PrP interaction to the onset and disease progression in synucleinopathies makes it an enticing therapeutic target, using a variety of possible approaches such as immunotherapy, anti-sense oligonucleotides, or small molecules.

AUTHOR CONTRIBUTIONS

Tuane C. R. G. Vieira: Conceptualization; funding acquisition; supervision; writing – original draft; writing – review and editing. **Caroline A. Barros:** Writing – original draft. **Renato Domingues:** Writing – original draft. **Tiago Fleming Outeiro:** Conceptualization; funding acquisition; project administration; supervision; writing – original draft; writing – review and editing.

ACKNOWLEDGMENTS

T.F.O. is supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy—EXC 2067/1-390729940, and by SFB1286 (B8). T.C.R.G.V. is supported by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) and by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

Tiago F. Outeiro is an Editor with the *Journal of Neurochemistry*, and the Editor of the current special issue, but played no role in the peer review of this manuscript.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jnc.15992.

DATA AVAILABILITY STATEMENT

Not applicable.

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How to cite this article: Vieira, T. C. R. G., Barros, C. A., Domingues, R., & Outeiro, T. F. (2023). PrP meets alpha-synuclein: Molecular mechanisms and implications for disease. *Journal of Neurochemistry*, *00*, 1–15. https://doi.org/10.1111/jnc.15992