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# PrP<sup>C</sup> as a Transducer of Physiological and Pathological Signals

Jessica D. Panes<sup>1†</sup>, Paulina Saavedra<sup>1,2†</sup>, Benjamin Pineda<sup>1</sup>, Kathleen Escobar<sup>1,2</sup>, Magdalena E. Cuevas<sup>1</sup>, Gustavo Moraga-Cid<sup>1</sup>, Jorge Fuentealba<sup>1</sup>, Coralía I. Rivas<sup>2</sup>, Human Rezaei<sup>3,4,5</sup> and Carola Muñoz-Montesino<sup>1\*</sup>

<sup>1</sup> Departamento de Fisiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile,

<sup>2</sup> Departamento de Fisiopatología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile,

<sup>3</sup> Virologie et Immunologie Moléculaires (VIM), Institut National de Recherche pour l'Agriculture, l'Alimentation et

l'Environnement (INRAE), Jouy-en-Josas, France, <sup>4</sup> Université de Versailles Saint-Quentin-en-Yvelines (UVSQ), Versailles,

France, <sup>5</sup> Université Paris-Saclay, Jouy-en-Josas, France

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### \*Correspondence:

Carola Muñoz-Montesino  
carmunozm@udec.cl

<sup>†</sup> These authors have contributed  
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After the discovery of prion phenomenon, the physiological role of the cellular prion protein (PrP<sup>C</sup>) remained elusive. In the past decades, molecular and cellular analysis has shed some light regarding interactions and functions of PrP<sup>C</sup> in health and disease. PrP<sup>C</sup>, which is located mainly at the plasma membrane of neuronal cells attached by a glycosylphosphatidylinositol (GPI) anchor, can act as a receptor or transducer from external signaling. Although the precise role of PrP<sup>C</sup> remains elusive, a variety of functions have been proposed for this protein, namely, neuronal excitability and viability. Although many issues must be solved to clearly define the role of PrP<sup>C</sup>, its connection to the central nervous system (CNS) and to several misfolding-associated diseases makes PrP<sup>C</sup> an interesting pharmacological target. In a physiological context, several reports have proposed that PrP<sup>C</sup> modulates synaptic transmission, interacting with various proteins, namely, ion pumps, channels, and metabotropic receptors. PrP<sup>C</sup> has also been implicated in the pathophysiological cell signaling induced by  $\beta$ -amyloid peptide that leads to synaptic dysfunction in the context of Alzheimer's disease (AD), as a mediator of A $\beta$ -induced cell toxicity. Additionally, it has been implicated in other proteinopathies as well. In this review, we aimed to analyze the role of PrP<sup>C</sup> as a transducer of physiological and pathological signaling.

**Keywords:** PrP, A $\beta$ , PrP<sup>C</sup> signaling, PrP<sup>C</sup> role, PrP<sup>C</sup> in CNS, Alzheimer's disease

## INTRODUCTION

Prion was first proposed by Stanley Prusiner in 1982 as an infectious protein. This occurred in the context of a group of rare encephalopathies of unknown etiology in sheep and goats, characterized by abnormal trembling termed "scrapie" (Prusiner, 1982). Based on the experiments of ultraviolet irradiation of brain extracts of infected mice, a novel infectious component of low molecular weight was observed which did not depend on canonical transmission by nucleic acids and exhibited replicative and infective capacity (Alper et al., 1967; Griffith, 1967). Later, this infectious particle was isolated and corresponded to a 27–30 kDa protein, which was devoid of nucleic acids, and it was resistant to digestion by proteinase K, which was named "prion" (Bolton et al., 1982; Prusiner, 1982).

Later, several studies revealed that the ability of prion to propagate was related to an abnormally folded variant of prion protein (PrP), which is naturally expressed in mammals (Prusiner, 1982;

Collinge, 2001). In this context, normally folded  $\alpha$ -helix-enriched cellular prion protein (PrP<sup>C</sup>) can be converted into a scrapie protease-resistant form of PrP (PrP<sup>Sc</sup>), requiring a cascade of conformational changes to form  $\beta$ -sheet-enriched conformation. Interestingly, PrP<sup>Sc</sup> can propagate its own altered conformation using PrP<sup>C</sup> as a substrate, in a template replication process (Griffith, 1967; Lansbury, 1994).

PrP<sup>C</sup> is highly expressed in different neuronal and astrocytic cells of several central nervous system (CNS) areas, namely, amygdala, cerebellum, hypothalamus, occipital lobe, prefrontal cortex, and spinal cord (Su et al., 2004; Castle and Gill, 2017). It is also moderately or poorly expressed in non-neuronal cells, such as immune system, and endothelial and epithelial cells of colon, uterus, ovary, thyroid, and small intestine (Isaacs et al., 2006; Petit et al., 2013). During embryonic development, the high levels of Prnp messenger RNA (mRNA) have also been found in the CNS and peripheral nervous system (PNS) (Manson et al., 1992; Beringue et al., 2003; Lima et al., 2007; Castle and Gill, 2017).

PrP is a key mediator in several toxicity pathways in some neurodegenerative diseases (NDs), such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) (Prusiner, 2012; Urrea et al., 2017). In this review, we have focused on summarizing the current knowledge of PrP<sup>C</sup> as a sensor and key mediator of physiological significance, its role as a transducer in the amyloid cascade in AD, and its effect on other misfolding-related diseases.

Although PrP<sup>C</sup> has been implicated in synapse growth, neural plasticity, and memory, a unified variant of its on-target sites is still unknown. In fact, its mechanisms of action have been the subject of intense research for almost three decades. Despite this, there are fundamental questions that are yet to be solved: (1) What is the biological consequence of the association of PrP<sup>C</sup> with normal protein folding process? (2) What is the physiological role of PrP<sup>C</sup> interaction with channels? (3) How do PrP species act in association with other misfolded proteins? Based on the current knowledge of the function of PrP<sup>C</sup>, we have reviewed the physiological and pathological roles of PrP<sup>C</sup> signaling on synaptic function, providing a new angle to the putative role of PrP in health and disease.

## STRUCTURE, PROCESSING, AND FUNCTION OF PrP

### Structural Biology of PrP<sup>C</sup>

PrP<sup>C</sup> is encoded by *PRNP* gene, located in chromosome 20 (in humans) or in chromosome 2 (in mice) (Chesebro et al., 1985; Sparkes et al., 1986). PrP<sup>C</sup> is a 210-residue glycoprotein attached to the cell surface by glycosylphosphatidylinositol (GPI) anchor (231–253 residues) (Stahl et al., 1987). Within the plasma membrane, PrP<sup>C</sup> is found at lipid rafts (also known as microdomains), enriched in cholesterol and sphingolipids (Simons and Gerl, 2010; Botto et al., 2014; Martellucci et al., 2020). Human PrP genomic cluster also contains the homologous genes *PRND* and *PRNT* of 55 kb, where *PRND* encodes for a Doppel (Dpl) protein of 179 residues and *PRNT* encodes three mRNA by alternative splicing, expressed exclusively in the testis

(Premzl and Gamulin, 2007). PrP genomic family member also includes Shadoo protein, encoded by *SPRN* gene and located in the human chromosome 10 (Ciric and Rezaei, 2015).

The PrP<sup>C</sup> first moiety corresponds to a highly positively charged polybasic N-terminal region that is intrinsically disordered and flexible (Beland and Roucou, 2012). Some functions of the N-terminal domain are associated with protein-protein interactions, synaptic transmission, neuroprotection, and Cu<sup>2+</sup>- or Zn<sup>2+</sup>-mediated modulation (Beland and Roucou, 2012; Turnbaugh et al., 2012; Martellucci et al., 2020). Particularly, N-terminal PrP<sup>C</sup> contains a signal peptide (1–22 residues) and four functional regions, namely, two positively charged clusters (CC1 and CC2), an octarepeat (OR), and a hydrophobic domain (HD) (Beland and Roucou, 2012; **Figure 1**).

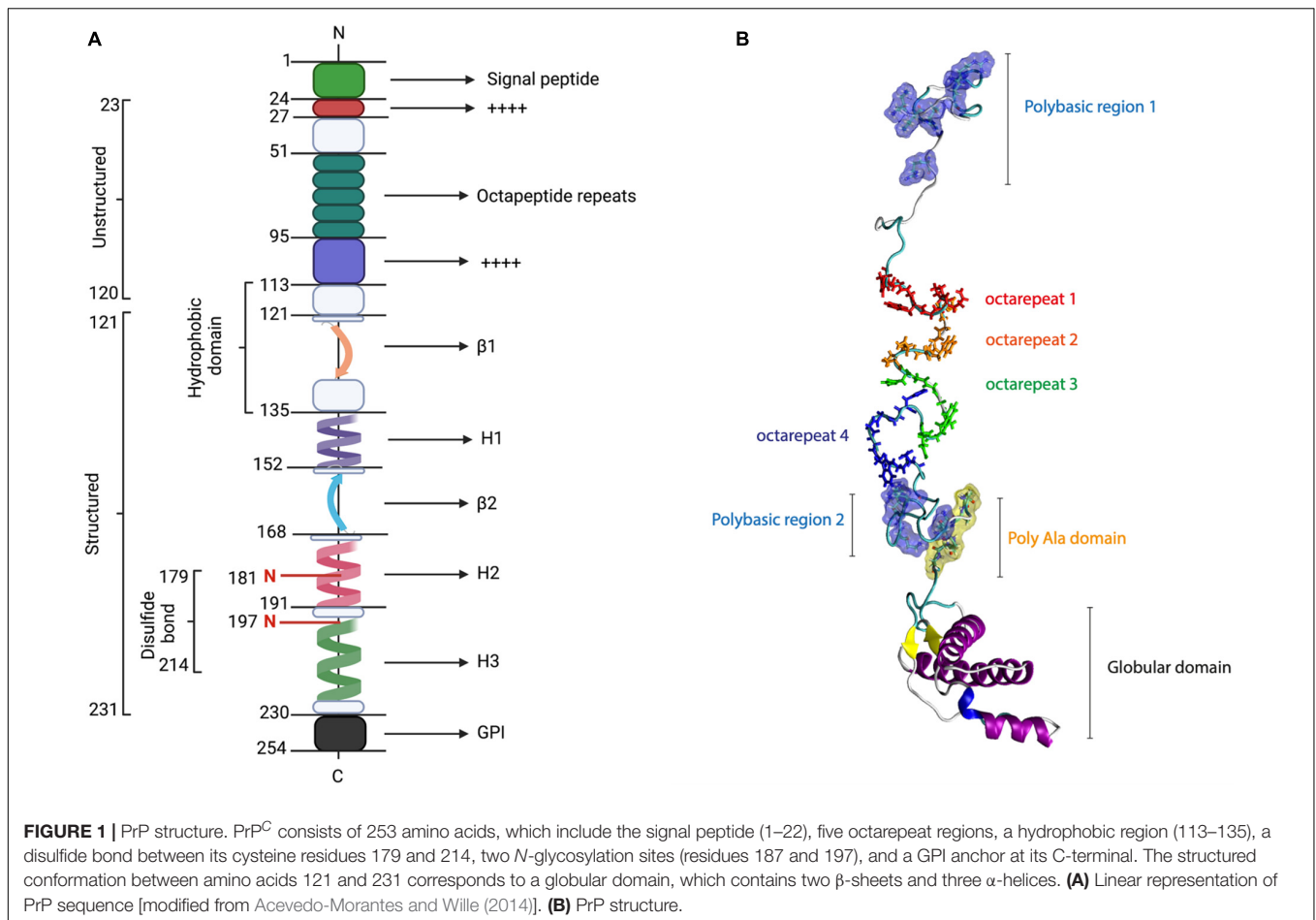
The PrP<sup>C</sup> CC1 domain (23–28 residues) has been associated with myelin homeostasis and PrP<sup>C</sup>  $\alpha$ -folding stability (Martinez et al., 2015; Kuffer et al., 2016). The OR region (51–91 residues) consists of five octarepeat sequence repeats (PHGGGWGQ), enriched with glycine and histidine (His) residues, which contains several Cu<sup>2+</sup>- and Zn<sup>2+</sup>-binding units (Beland and Roucou, 2012; Wu et al., 2017). Currently, this region has been related to PrP<sup>C</sup> endoproteolysis, Cu<sup>2+</sup> metabolism, and the initial steps of PrP<sup>C</sup>–PrP<sup>Sc</sup> conversion (Lau et al., 2015).

In contrast, the PrP<sup>C</sup> CC2 region (100–109 residues) has been associated with lipid membranes, PrP<sup>C</sup> processing, and PrP<sup>C</sup> biogenesis (Kim et al., 2001; Wang et al., 2010; Martinez et al., 2015). In the core of the primary structure of PrP<sup>C</sup>, it has been described in the HD region (111–130 residues), which seems to have a neuroprotective role against neurotoxicity and myelotoxicity (Beland and Roucou, 2012; Gavin et al., 2020). Interestingly, HD seems to be relevant for the stabilization of PrP<sup>C</sup> homodimers, contributing to the prevention of prion conversion (Warwicker, 2000; Engelke et al., 2018).

As depicted in **Figure 1**, the C-terminal globular domain of PrP<sup>C</sup> is composed of three  $\alpha$ -helix structures, two antiparallel  $\beta$ -sheets, and a GPI anchor (Heske et al., 2004; Sarnataro et al., 2017). Additionally,  $\alpha$ -helices 2 and 3 are connected by a disulfide bond between cysteines 179 and 214, contributing to the stability of PrP<sup>C</sup>-folded state (Biasini et al., 2012). Another important function that was proposed for the C-terminal domain of PrP<sup>C</sup> is its neuroprotective activity against excitotoxicity mediated by Cu<sup>2+</sup> coordination with N-terminal (Heske et al., 2004; Schilling et al., 2020). The globular domain of PrP is highly conserved among mammals (Salamat et al., 2013) and exhibits high structural similarity with the Dpl, sharing 25% identity (Ciric and Rezaei, 2015). This region is central in the conversion process; however, it allows certain changes, namely, insertions and deletions, in the C-terminal portion of H<sub>2</sub> without affecting the conversion process (Salamat et al., 2012, 2013; Ciric and Rezaei, 2015; Munoz-Montesino et al., 2016, 2017).

### Sorting and Processing of PrP<sup>C</sup>

Since the late 1990s, posttranslational modifications (PTMs) have been recognized as the main regulators of PrP<sup>C</sup> biosynthesis. The trafficking of PrP<sup>C</sup> precursor protein (253 residues) to the plasma membrane starts with the internalization into the endoplasmic reticulum (ER) by an N-terminal signal peptide



(Heller et al., 2003; Chakrabarti et al., 2009; Miranzadeh Mahabadi and Taghibiglou, 2020). After translocation of ER, several PTM occurs to allow PrP<sup>C</sup> folding (23–231 residues), namely, C-terminal hydrophobic segment cleavage, C-terminal GPI anchor attachment, and the addition of different patterns of *N*-linked glycosylation (181 and 197 residues in humans, and 180 and 196 residues in mice), which can lead to a diglycosylated, monoglycosylated, or unglycosylated species (Choi, 1992; Zuegg and Gready, 2000; Miranzadeh Mahabadi and Taghibiglou, 2020).

Not all newly synthesized PrP<sup>C</sup> is translocated into the plasma membrane. There are two transmembrane (TM) PrP<sup>C</sup> topologies that are retained into ER or Golgi for proteasomal degradation, namely, the *N*-transmembrane (NtmPrP<sup>C</sup>) and cytosol transmembrane (CtmPrP<sup>C</sup>) (Hegde et al., 1998; Sarnataro et al., 2017). There are no precise physiological or pathological functions for NtmPrP<sup>C</sup> up to date (Westergard et al., 2007; Miranzadeh Mahabadi and Taghibiglou, 2020). In contrast, CtmPrP<sup>C</sup> has been associated with some neurodegenerative pathways, namely, PrP<sup>Sc</sup> accumulation, ER stress, cell death, and neurodegeneration (Hegde et al., 1998; Crozet et al., 2008; Gavin et al., 2020).

Later, PrP<sup>C</sup> is transported through the Golgi apparatus to the *trans*-Golgi network (TGN), where several PTMs are found to be translocated finally to the plasma membrane,

where it remains attached by its GPI anchor (Biasini et al., 2012; Miranzadeh Mahabadi and Taghibiglou, 2020). PrP<sup>C</sup> traffics through endocytic recycling compartment, mediated by clathrin-dependent mechanism, where it can be sorted in the plasma membrane for recycling or endolysosomal pathway for degradation (Chakrabarti et al., 2009; Marijanovic et al., 2009; Yim et al., 2015).

The proteolytic processing of PrP<sup>C</sup> has been the focus of numerous studies due to physiological or pathological significance of the cleavages, which is still uncertain. Normally, PrP<sup>C</sup> can be processed mainly by two proteolytic pathways. First, PrP<sup>C</sup>  $\alpha$ -cleavage (that occurs at residues 110–111 or 111–112) generates a soluble  $\sim$ 11 kDa fragment from PrP<sup>C</sup> N-terminal domain (N1), as well as a  $\sim$ 16 kDa fragment from PrP<sup>C</sup> C-terminal region which remains attached to the plasma membrane (C1) (Mange et al., 2004; Liang and Kong, 2012). Second, PrP<sup>C</sup>  $\beta$ -cleavage releases a longer fragment that remains attached to the membrane (C2) of  $\sim$ 18 kDa and a  $\sim$ 9 kDa fragment from PrP<sup>C</sup> N-terminal domain (N2) (Biasini et al., 2012; Castle and Gill, 2017).

Although the regulatory role for C1 fragment production is unresolved, it seems that it negatively modulates key steps of PrP conversion process, namely, misfolding, replication, and fibrillization (Westergard et al., 2011; Campbell et al., 2013).

Nevertheless, under experimental conditions, C1 lacking the C-terminal portion of H<sub>2</sub> can be converted into a C1 prion by full-length spontaneous prion harboring the same deletion (Munoz-Montesino et al., 2020). Regarding C2 fragment, data strongly suggest that its accumulation would be a key product of the PrP<sup>C</sup> processing in prion replication (Dron et al., 2010). It is likely that C2 represents an important PrP<sup>Sc</sup> phenotype-contributing factor during prion disease (Dron et al., 2010).

## PHYSIOLOGICAL FUNCTIONS OF PRP<sup>C</sup>

Although the precise function of PrP<sup>C</sup> at the cell surface is not completely understood, some researchers have proposed that it might be important in the nervous system, namely, the formation of synapses, neuronal viability, neuronal excitability, cell motility and neuronal growth, antiapoptotic effect, neurite adhesion, stress sensibility, and calcium homeostasis (Herms et al., 2000; Pantera et al., 2009; Carulla et al., 2011; Park et al., 2015; Wulf et al., 2017; Prado et al., 2020). Additionally, PrP<sup>C</sup> has been related to the immune system, namely, T-cell activation, the release of reactive oxygen species (ROS), monocyte maturation, and macrophage phagocytic activity (Isaacs et al., 2006; Miranzadeh Mahabadi and Taghibiglou, 2020). PrP<sup>C</sup> also participates in several signaling pathways that regulate innate immunity, namely, Akt, ERK-1/2, and NF-κB (Jeon et al., 2013).

To characterize the physiological function of PrP<sup>C</sup>, the initial strategy was to develop PrP<sup>C</sup> knockout (KO) mice. The first KOs developed were called Zurich and Npu, both of which did not show marked phenotypes. In both animals, the transmission by prions was completely prevented since the substrate for prion conversion, PrP, was absent (Bueler et al., 1992; Manson et al., 1994). Later, new models, namely, Zurich II, Ngsk, and Rcm0, developed late ataxia due to degeneration of Purkinje neurons (Sakaguchi et al., 1996). In these models, overexpression of Dpl was observed and it would be this protein that causes the death of this type of neurons due to neurotoxicity and not due to the lack of PrP<sup>C</sup>. Likewise, it has been established *in vitro* that overexpression of Dpl is toxic only when PrP<sup>C</sup> is not expressed; therefore, an interaction between both proteins is suggested to mediate toxicity phenomena (Sakudo et al., 2005).

Likewise, PrP<sup>C</sup> modulates growth factor receptor (EGFR) function in regulating cell cycle and growth (Llorens et al., 2013). Another function reported for PrP<sup>C</sup> is protection against oxidative stress. It has been determined that in SH-SY5Y neuroblastoma cells in which PrP<sup>C</sup> was overexpressed, there was greater resistance to oxidative stress than cells expressing endogenous levels and that this protection would be given by the N-terminal portion of PrP<sup>C</sup> (Zeng et al., 2003).

Even though the metal-binding relevance to PrP<sup>C</sup> study represents a challenge, a large number of studies support that PrP<sup>C</sup> could be involved in copper homeostasis due to its N-terminal unstructured portion. Two main regions are involved in the copper-binding ability of PrP<sup>C</sup>: first is the highly conserved octarepeat (OR) region (residues 60–91), where the His residues can bind up to four copper ions with high affinity, and second is the so-called non-OR region (residues 92–111), where two

additional His residues are able to bind copper. This non-OR region is contiguous to a hydrophobic portion (residues 112–127) and is thought to be relevant during prion conversion (Giachin et al., 2015). Single His residue mutation in both OR and non-OR regions analyses has supported the idea of the critical role of copper-binding residues, suggesting also its role in regulating the function of PrP<sup>C</sup> in neuritogenesis and preserving the functional conformation of the protein, thus contributing to modulate prion conversion propensity (Nguyen et al., 2019). Therefore, copper binding might be relevant to both physiological and pathological roles of PrP<sup>C</sup>. Other roles associated with their interaction are endocytosis stimulation and trafficking, antioxidant effect, NMDA receptors modulation, and brain metal homeostasis (Salzano et al., 2019). Metal ion regulation in the CNS has also been related to NDs such as AD and PD (Salzano et al., 2019).

Finally, under physiological conditions in the nervous system, it has been reported that PrP<sup>C</sup> is mediating several functions such as cell growth, metal homeostasis, neuritic growth, the formation of lamellipodia, and synaptic transmission (Carulla et al., 2011; Llorens et al., 2013; Legname, 2017; Huang et al., 2018; Nguyen et al., 2019; Prado et al., 2020). The signaling pathways associated with PrP<sup>C</sup> neuronal growth-associated functions are achieved by its association with different proteins, such as NCAM and laminin, to promote neurite growth through the activation of Fyn kinase (Schmitt-Ulms et al., 2001; Santucci et al., 2005). Also, it was determined that PrP<sup>C</sup> participates in myelin homeostasis in Schwann cells through interaction with its N-terminal through residues 23–33 with the GPCR 126 receptor on the surface of these cells (Kuffer et al., 2016). The role of PrP<sup>C</sup> in neuronal function is further discussed in the subsequent sections.

## Role of PrP<sup>C</sup> in Neuronal Function From a Perspective of the Synaptic Transmission

The normal physiological functions and cell behavior of PrP<sup>C</sup>, namely, neurite outgrowth, synaptogenesis, synaptic function, and neuroprotection, are not yet well understood. PrP<sup>C</sup> has been associated with several intracellular signaling pathways that modulate neuronal signal transduction and it participates in the organization of physiological brain networks, such as neuronal excitability, neuroprotection, neuritogenesis, neurotrophic function, and neuronal plasticity (Linden et al., 2008; Carulla et al., 2015; Castle and Gill, 2017; Linden, 2017). However, to understand how PrP<sup>C</sup> can regulate synaptic plasticity by neuronal activity, it is necessary to study the functional interaction of PrP<sup>C</sup> with transporters, ion pumps, ion channels, and metabotropic receptors expressed in neuronal cell surface (Table 1). We thus approached PrP<sup>C</sup> modulation in two key processes, namely, action potentials (APs) and postsynaptic potentials (PSPs), that coordinate the correct functioning of neuronal performance and the generation of a nerve impulse.

## Role of PrP<sup>C</sup> in Action Potentials

Collinge et al. (1994) established the role of PrP on neuronal excitability by electrophysiological studies in hippocampal

**TABLE 1** | Summary of the main effects of PrP<sup>C</sup> in synaptic function.

Binding interaction	Model	Functional role	Proposed mechanism of action	References
VGCC $\alpha 2\delta$ -1 subunit	Tg PG14 mice (CGNs) Xenopus oocytes and mammalian tsA-201 cells	Glutamatergic neurotransmission Modulation of Ca <sup>2+</sup> currents	Promotes anterograde trafficking and secretory transport of VGCC channels to the cell membrane Downregulates CaV2.1/ $\beta 4/\alpha 2\delta$ -2 and CaV2.1/ $\beta 1b/\alpha 2\delta$ -1 channels in a GPI-anchoring form	Rutishauser et al., 2009; Senatore et al., 2012; Alvarez-Laviada et al., 2014
Kv4.2 DPP6 subunit	N2a, RK13, and HEK293T cells	Regulation of membrane excitability	Increases peak current amplitudes and the half-inactivation time of A-type K <sup>+</sup> currents Regulates faster recovery time from steady-state inactivation of Kv4.2 channel	Schmitt-Ulms et al., 2004; Mercer et al., 2013
NMDAR NR1/NR2B subunits	Tga20 knock-in mice (hippocampal neurons) Prnp <sup>0/0</sup> FVB/N and C57 mice	Neuroprotection Modulation of NMDAR activity	Downregulates NR2D subunits expression and S-nitrosylation of NMDAR Reduces glycine affinity, slows inactivation and current amplitudes of NMDAR	Khosravani et al., 2008a; Black et al., 2014; Gasperini et al., 2015; Huang et al., 2018
AMPA GluA2 and GluA4 subunits	Cultured astrocytes from PrP <sup>-/-</sup> mice SH-SY5Y and N2a cells Hippocampal neurons from PrP <sup>C</sup> -overexpressed mice Tg PG14 and CJD mice	Glutamate-dependent lactate release Zinc uptake Non-affected AMPAR activity Neuronal survival	Regulates the MCT1-associated lactate transport and Na <sup>+</sup> /K <sup>+</sup> pump astrocytic activity Zinc-sensitive tyrosine phosphatase activity NA Modulates secretory trafficking of AMPAR GluA2 subunit	Kleene et al., 2007; Khosravani et al., 2008b; Watt et al., 2012; Huang et al., 2018; Ghirardini et al., 2020
KARs GluR6/7 and PSD95 subunits	Prnp <sup>0/0</sup> mice N2a cells Jnk3 <sup>0/0</sup> mice	Neuroprotection against KA toxicity Neuronal survival	Regulates GluR6 and GluR7 mRNA levels Modulates KA-mediated neurotransmission Regulates PSD95/GluR6 complex	Rangel et al., 2007; Carulla et al., 2011, 2015
$\alpha 7nAChR$ /ST11 complex	Hippocampal neurons HEK293 cells ZW 13-2 and Zpl 3-4 cell lines from Prnp <sup>-/-</sup> mice	Ca <sup>2+</sup> homeostasis, neurogenesis, and neuroprotection	Modulates positively $\alpha 7nAChR$ activity PKA activity and ERK1/2 phosphorylation Regulates $\alpha 7nAChR$ expression levels	Beraldo et al., 2010; Jeong and Park, 2015

PrP<sup>C</sup>, cellular prion protein; CGNs, cerebellar granule neurons; CaV, voltage-gated Ca<sup>2+</sup> channels; DPP6, dipeptidyl aminopeptidase-like protein 6; DPP6, dipeptidyl aminopeptidase-like protein 6; Kv, voltage-dependent K<sup>+</sup> channels, FVB/N, Friend virus B-type susceptibility-NIH; AMPAR,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; MCT1, astroglial monocarboxylate transporter 1; CJD, Creutzfeldt-Jakob disease; KA, kainate; KARs, kainate receptor; N2a, murine neuroblastoma cell line Neuro2a; JNK3, c-Jun N-terminal kinase 3; PSD-95, postsynaptic density protein 95;  $\alpha 7nAChR$ , nicotinic acetylcholine receptor; ST11, stress-inducible protein 1; PKA, cAMP-dependent protein kinase 1; ERK1/2, extracellular signal-regulated kinase 1 and 2.

pyramidal neurons and Purkinje cells, from non-transgenic (N-Tg) mice and conditional PrP<sup>C</sup>-null mice (Prnp0/0). Interestingly, the histopathological evaluation of Prnp0/0 did not exhibit significant variations with N-Tg mice, but it showed alterations on feedback mechanisms controlling frequency and patterning of neuronal firing, such as input resistance (Rinp), Ca<sup>2+</sup>-activated K<sup>+</sup> current (IAHP), and afterhyperpolarization (AHP) current (Collinge et al., 1994; Collinge et al., 1996; Herms et al., 2001; Mallucci et al., 2002).

One of the main modulators in the generation and shaping of APs is voltage-dependent calcium channels (VGCCs or CaV) (Llinas et al., 1976; Campiglio and Flucher, 2015). Electrophysiological and immunohistochemical studies have shown that PrP<sup>C</sup> is able to maintain neuronal excitability at the presynaptic level. This is achieved by stabilization and interaction with  $\alpha 2\delta$ -1 auxiliary subunit of VGCC channels in a GPI anchor-dependent manner (Table 1; Rutishauser et al., 2009; Senatore et al., 2012; Alvarez-Laviada et al., 2014). Furthermore, co-expression of PrP with different Ca<sup>2+</sup> channel subunits in *Xenopus* oocytes and mammalian tsA-201 cells has shown that PrP is able to modulate the amplitude peak of Ca<sup>2+</sup> currents of the CaV2.1/ $\beta 4/\alpha 2\delta$ -2 and CaV2.1/ $\beta 1b/\alpha 2\delta$ -1 channels (Alvarez-Laviada et al., 2014). In contrast, in cerebellar granule neurons (CGN) of the transgenic mouse of PrP Tg (PG14), which synthesizes a misfolded mutant variant of PrP (PrPmut) that

is partially retained in the ER, it was observed that PrPmut can impair  $\alpha 2\delta$ -1 auxiliary subunit anterograde trafficking, reducing intracellular Ca<sup>2+</sup> influx and glutamate transmission into the synaptic cleft (Senatore et al., 2012). Furthermore, PrP<sup>C</sup> modulates neuronal membrane excitability, synaptic integration of voltage threshold, and the repolarization process of the APs, mediated by their functional interaction with the Kv4.2 (voltage-gated K channels)/DPP6 (dipeptidyl aminopeptidase-like protein 6) complex at the neuronal cell surface (Schmitt-Ulms et al., 2004; Kim et al., 2008; Mercer et al., 2013). Electrophysiological studies in HEK293T cells transiently transfected with the Kv4.2/DPP6 channel complex have shown that PrP<sup>C</sup> is able to increase the amplitude peak and depolarizing potential of A-type K<sup>+</sup> currents, as well as it shifts the activation curve of the Kv4.2 channels to more depolarized potentials in a DPP6-dependent form (Mercer et al., 2013). Further studies are needed to understand the link between PrP<sup>C</sup>, its misfolding, and the neuronal activity-dependent signaling pathways during the APs.

### Role of PrP<sup>C</sup> in Postsynaptic Potentials

PrP<sup>C</sup> also participates in the regulation of excitatory postsynaptic responses through its functional interaction with ionotropic receptors, namely, N-methyl-D-aspartate receptor (NMDARs) (Khosravani et al., 2008a; You et al., 2012),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptors

(Watt et al., 2012; Chater and Goda, 2014), kainate receptor (KARs) (Carulla et al., 2011), and  $\alpha 7$  nicotinic acetylcholine receptors ( $\alpha 7$ nAChRs) (Zanata et al., 2002; Beraldo et al., 2010; Roffe et al., 2010; **Table 1**).

Increasing studies indicate that PrP<sup>C</sup> would be a key mediator in the maintenance of glutamatergic synapses, mediated by their interaction with NR1 and NR2 subunits of NMDAR (Khosravani et al., 2008a; You et al., 2012; Gasperini et al., 2015). It was observed that PrP<sup>C</sup> ablation induced an overexpression and S-nitrosylation of the NR2A and NR2B subunits of NMDAR, altering its kinetic properties. PrP<sup>C</sup> ablation induced a slow inactivation of the channel triggering an abnormal increase in neuronal excitability (Gasperini et al., 2015). Meanwhile, overexpression of mouse PrP<sup>C</sup> showed decreased activity of NMDAR (Maglio et al., 2004; Khosravani et al., 2008a; Gasperini et al., 2015; Huang et al., 2018). Additionally, recent studies have shown that the neuroprotective effects of PrP<sup>C</sup> associated with downregulation of NMDAR would occur in a Cu<sup>2+</sup>-dependent manner (Gasperini et al., 2015; Huang et al., 2018). More studies are needed to establish the interaction sites of PrP<sup>C</sup> in the modulation of NMDAR activity.

Regarding AMPA receptors, *in vitro* co-immunoprecipitation studies also revealed interactions with PrP<sup>C</sup> (Kleene et al., 2007; Watt et al., 2012; Huang et al., 2018). Interestingly, it has been observed that the increase in the formation of PrP<sup>C</sup>/AMPA complex could exert neuroprotection in a Cu<sup>2+</sup>- and Zn<sup>2+</sup>-dependent manner, as well as AMPA-ergic activity (Watt et al., 2012; Huang et al., 2018). However, PrP<sup>C</sup> modulation does not induce significant changes in the amplitude or channel kinetics nor the long-term depression (LTD) maintenance (Khosravani et al., 2008a; Huang et al., 2018). Remarkably, the mutant variant of PrP could exert excitotoxicity mediated by intracellularly retained GluA2 AMPAR subunit (Ghirardini et al., 2020).

It has been postulated that PrP<sup>C</sup> has a neuroprotective function in association with KARs against neurotoxicity induced by kainite (KA), which induces neurodegeneration in presynaptic terminals (Carulla et al., 2011). Additionally, *in vivo* and *in vitro* evidence in Prnp0/0 mice indicated that PrP<sup>C</sup> can also regulate synaptic transmission and exert neuroprotection against KA toxicity, in a GPI anchoring-dependent manner (Rangel et al., 2007; Carulla et al., 2015). More studies are needed to determine the direct action of PrP<sup>C</sup> in channel kinetics and KARs activity, mediated by postsynaptic density protein 95 (PSD95) modulation.

Another postulated mechanism by which PrP<sup>C</sup> would exert neuroprotection and promote neuritogenesis is related to its association with  $\alpha 7$ nAChRs/stress-inducible protein 1 (STI1) complex at the cell membrane (Beraldo et al., 2010). Effectively, in several neuron cell lines, it has been observed that PrP<sup>C</sup> can upregulate several neuroprotective pathways, such as autophagic flux cAMP-dependent protein kinase 1 (PKA), and extracellular signal-regulated kinase 1 and 2 (ERK1/2) pathways, in a  $\alpha 7$ nAChRs-dependent manner (Beraldo et al., 2010; Jeong and Park, 2015).

The overall data suggest that PrP<sup>C</sup> would be acting as a new player in the regulation of glutamatergic and cholinergic

neurotransmission. However, further research is needed to identify regions involved in the association of ionotropic receptors and PrP<sup>C</sup>, as well as the consequences of its disruption in the synaptic neurotransmission in a pathological context.

## ROLE OF PrP<sup>C</sup> IN PATHOLOGY

### PrP<sup>C</sup> and Alzheimer

Alzheimer's disease is a progressive disorder associated with cerebral cortex atrophy and irreversible loss of cortical neurons (Musiek and Schindler, 2013). AD is mainly characterized by an accumulation of amyloid- $\beta$  (A $\beta$ ) plaques and phosphorylated Tau protein neurofibrillary tangles. The major plaque component is A $\beta$  peptide made of 39–43 amino acids, which are derived from the amyloid precursor protein (APP) (Selkoe, 2001; Walsh and Selkoe, 2007). A $\beta$  monomers are not toxic and do not interfere with the synapses, whereas small oligomers and larger aggregates are most likely to be the most toxic species, impairing synaptic plasticity (Legname and Scialo, 2020).

Several studies have related PrP with AD (Kellett and Hooper, 2009); however, the mechanism by which PrP affects the progression of the disease is not clear. Also, there is still controversy regarding whether or not PrP<sup>C</sup> is required for A $\beta$  toxicity (Legname and Scialo, 2020). Therefore, we discussed the evidence for interaction of PrP<sup>C</sup> and A $\beta$  and its role in mediating A $\beta$  toxicity.

### Interaction of PrP<sup>C</sup> and A $\beta$

A $\beta$  oligomers (A $\beta$ Os)-induced neuronal toxicity is thought, at least partly, to be mediated by putative A $\beta$  receptors. Among them, PrP<sup>C</sup> has emerged as an important potential receptor, due to its high affinity to the oligomeric form of the peptide (Laurén et al., 2009; Smith et al., 2019; Legname and Scialo, 2020). A cloning cDNA screening from a mouse brain library in order to find a protein that binds to A $\beta$ Os (A $\beta$ 1-42) found that the only high-affinity binding protein was PrP<sup>C</sup>, an observation that has been further supported by other studies (Laurén et al., 2009; Corbett et al., 2020). In fact, in a systematic comparison of reported A $\beta$  receptors, only PrP<sup>C</sup>, Nogo receptor 1 (NgR1), and leukocyte immunoglobulin-like receptor subfamily member 2 (LilrB2) showed direct binding to synthetic A $\beta$  assemblies. Interestingly, binding with human AD brains-derived soluble A $\beta$ Os revealed strong affinity only for PrP<sup>C</sup>, with a weak affinity for NgR1 and no detectable affinity for LilrB2 (Smith et al., 2019). Therefore, PrP<sup>C</sup> is most likely an A $\beta$ -binding receptor.

In contrast to what was observed between PrP<sup>C</sup> and A $\beta$ Os, experiments performed *in vitro* showed low-affinity interactions with A $\beta$  monomers (Chen et al., 2010; Fluharty et al., 2013; Corbett et al., 2020). Solid-phase assays showed that there is neither interaction of monomeric A $\beta$ 1-42 with PrP23–231 nor full-length PrP<sup>C</sup> (Corbett et al., 2020). However, immunoassay studies have revealed that PrP<sup>C</sup> 23–39 and 93–119 can interact with monomeric A $\beta$ 1-42 (Kang et al., 2013). Reported sites of interaction between PrP<sup>C</sup> and different A $\beta$  species are summarized in **Table 2**.

**TABLE 2** | A $\beta$ -PrP<sup>C</sup> interaction sites.

Binding site of PrP <sup>C</sup>	A $\beta$ species	Model	Cellular functions	References
95–110	A $\beta$ O <sub>s</sub> (~500 kDa)	PrP <sup>C</sup> -expressing COS-7 cells	Promotes LTP impairment, cell death, and cognitive impaired	Laurén et al., 2009; Gimbel et al., 2010;
NA	Brain-derived A $\beta$	Prnp <sup>-/-</sup> and C57Bl6 slices		
96–104	A $\beta$ O <sub>s</sub> (dimers)	Prnp <sup>-/-</sup> and APP <sup>Swe</sup> /PSEN1 $\Delta$ E9 slices	Barry et al., 2011; Nicoll et al., 2013;	Dohler et al., 2014;
NA	A $\beta$ protofibrils	A $\beta$ -containing AD brain		
N-terminus	A $\beta$ O <sub>s</sub> (300 and 158 kDa)	Prnp <sup>-/-</sup> and C57Bl6 slices	Kostylev et al., 2015;	Corbett et al., 2020
91–231	A $\beta$ O <sub>s</sub> (HMW assemblies)	Synthetic and A $\beta$ -containing AD brain		
	A $\beta$ O <sub>s</sub> (EC <sub>50</sub> ~30 nM)	Tg2576 mice and A $\beta$ -containing AD brains Prnp <sup>-/-</sup> and C57BL/6J slices		

PrP<sup>C</sup>, cellular prion protein; A $\beta$ O<sub>s</sub>, oligomers of A $\beta$  peptide; LTP, long-term potentiation; Swe, Swedish mutation; PSEN1, Presenilin-1, HMW, high-molecular-weight assemblies; AD, Alzheimer's disease.

Regarding the binding site in PrP<sup>C</sup> for A $\beta$ O<sub>s</sub>, it was shown that the unstructured N-terminal domain was relevant for this interaction (Laurén et al., 2009). In fact, when anti-PrP antibodies were used to interfere with the interaction, only 6D11 (which binds to amino acids 93–109 in mouse PrP) blocked the binding between A $\beta$  assemblies and PrP<sup>C</sup> with an IC<sub>50</sub> of 1 nM (Laurén et al., 2009). In addition, the deletion of a similar region (95–105) impaired A $\beta$  binding to PrP<sup>C</sup> (Laurén et al., 2009). Another site reported for this binding was the N-terminal basic amino acids 23–27 (KKRPK) in PrP (Legname and Scialo, 2020).

### PrP<sup>C</sup> as a Receptor of A $\beta$ Toxicity

Protein misfolding and aggregation of A $\beta$  peptide are key events in the onset of AD, especially A $\beta$ O<sub>s</sub>, due its capacity to associate with the cell membrane and induce excitotoxicity (Puzzo et al., 2017; Cline et al., 2018). The main neurotoxic effects described for A $\beta$ O<sub>s</sub> in AD are membrane disruption, synaptic failure, impaired LTP, and memory loss (Lambert et al., 1998; Cline et al., 2018). However, specific binding transducers of A $\beta$ O<sub>s</sub> signals that mediate its neurotoxic effects are not yet clearly defined. Several works have postulated different interacting partners for A $\beta$  assemblies in the cell membrane, namely, NMDAR (Rammes et al., 2018), APP (Puzzo et al., 2017), NgR1, nAChR, and PrP<sup>C</sup> (Fabiani and Antollini, 2019; Smith et al., 2019; Zhang et al., 2019).

As mentioned earlier, PrP<sup>C</sup> has been proposed as a high-affinity physiological receptor for soluble A $\beta$ O<sub>s</sub> [see reviews Linden (2017) and Wiatrak et al. (2021)]. At present, in different animal AD models as well as in patients with AD, it has been established that PrP<sup>C</sup> could be one of the best specific binding partners for A $\beta$ O<sub>s</sub>-mediated inhibition of LTP and cognitive defects in the early stages of AD (Kostylev et al., 2015; Smith and Strittmatter, 2017; Smith et al., 2019; Corbett et al., 2020). With this knowledge, it has been proposed that PrP<sup>C</sup> would play an important role in the onset of AD, occurring before clinical symptoms, such as movement and cognitive impairments associated with the late stages of the disease.

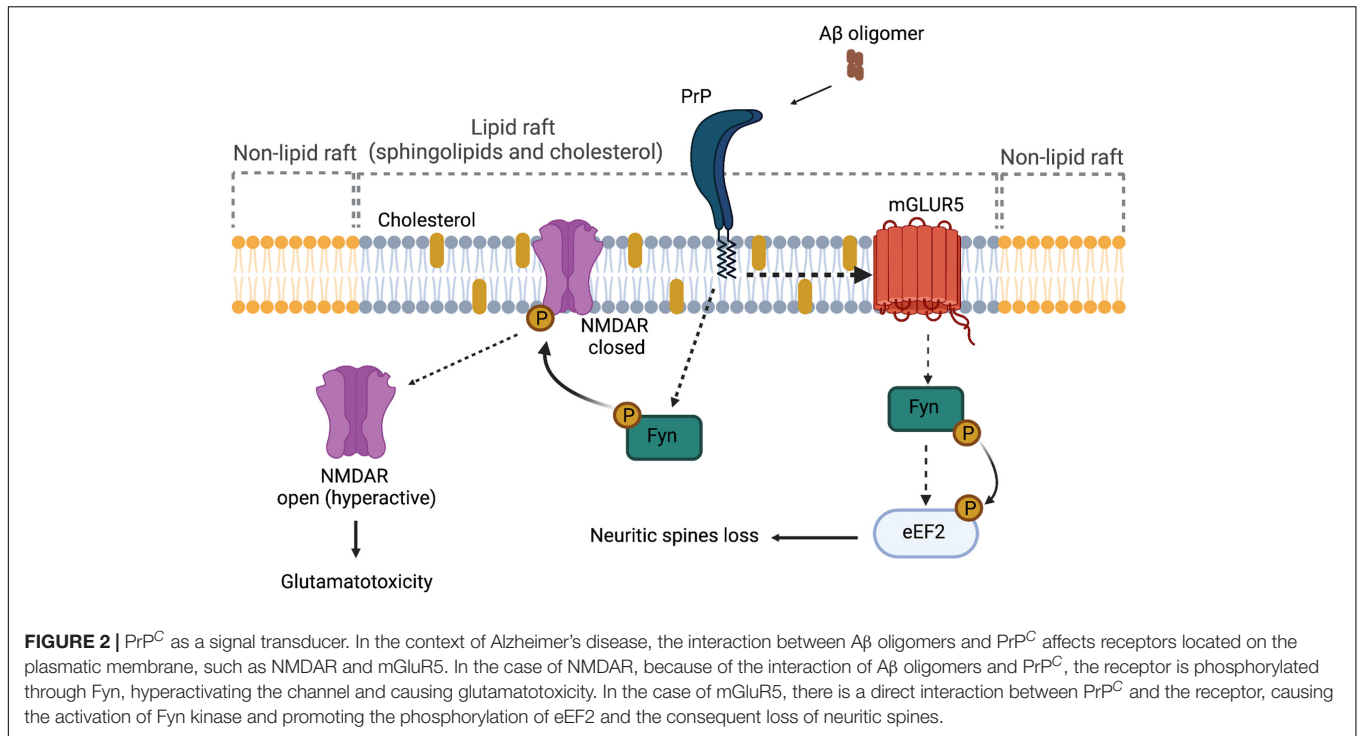
Laurén et al. (2009) have demonstrated that PrP<sup>C</sup> is a high-affinity receptor for A $\beta$ O<sub>s</sub>, being amino acids 95–110 of PrP<sup>C</sup> involved in this interaction (Laurén et al., 2009). Solid-phase and ELISA-like assays showed further associations between A $\beta$ O<sub>s</sub> and PrP<sup>C</sup> (EC<sub>50</sub> ~30 nM) (Corbett et al., 2020). In

cellular and animal models of A $\beta$  toxicity, PrP<sup>C</sup> was able to mediate impairment of synaptic plasticity, alteration in calcium transients, and reduction in the levels of synaptophysin (Riek et al., 1996; Laurén et al., 2009; Barry et al., 2011; Peters et al., 2015). Furthermore, these alterations can be rescued using antibodies that block the oligomer-binding site of A $\beta$  in PrP<sup>C</sup> (6D11) (Riek et al., 1996; Laurén et al., 2009; Barry et al., 2011; Peters et al., 2015). Regarding the mechanism by which PrP<sup>C</sup> exerts its role as a receptor, it has been proposed that A $\beta$ O<sub>s</sub> binding to PrP induces activation of Fyn, a Src kinase (SRK), through an undetermined TM partner (**Figure 2**; Malaga-Trillo and Ochs, 2016). After its activation, fyn phosphorylates NMDA receptor, which becomes transiently over-activated, producing excitotoxicity (Malaga-Trillo and Ochs, 2016). Fyn was already known to be relevant in the pathogenesis of AD, because it performs Tau phosphorylation. Tau is an axonal microtubule-associated protein, and phosphorylated Tau is the main constituent of neurofibrillary tangles in AD, which mediates A $\beta$  toxicity at the post-synapse. The notion that A $\beta$ O-induced Tau phosphorylation is mediated by PrP<sup>C</sup> comes from assays in human and mice brain, as well as analyses in primary neuron cultures, which show that soluble A $\beta$  binds to a PrP<sup>C</sup>/Fyn complex and Prnp gene deletion uncouples A $\beta$ O<sub>s</sub> and the Fyn/tau axis (Larson et al., 2012). Besides Tau phosphorylation, SRKs are able to regulate the stability at the neuronal plasma membrane of several synapse-relevant proteins as adhesion proteins and receptors (e.g., NMDAR, AMPAR, and GABAR) (Malaga-Trillo and Ochs, 2016). Therefore, PrP<sup>C</sup>-Fyn interaction might be directly involved in the pathological characteristics of AD. The signal transduction pathway generated by the interaction between A $\beta$ O<sub>s</sub> and PrP<sup>C</sup> is depicted in **Figure 2**.

### Role of PrP in Other Neurodegenerative Disorders

Neurodegeneration caused by protein misfolding and aggregation is characterized by progressive neuronal dysfunction associated with deposition of insoluble aggregates from a misfolded protein (Legname and Scialo, 2020). As discussed earlier, in prion diseases, the appearance of PrP<sup>Sc</sup> assemblies is involved in this process. In the case of AD, we have mentioned that A $\beta$ O<sub>s</sub>, A $\beta$  fibrils and plaques, and Tau tangles appear in the brain of patients with AD and that certain of these species are





related to the neurotoxicity and neurodegeneration. In other proteinopathies, amyloids and deposits of other proteins, such as TAR DNA-binding protein 43 (TDP-43),  $\alpha$ -synuclein ( $\alpha$ -syn), and Tau, are found (Legname and Scialo, 2020; Scialo et al., 2021). In the following sections, how some of these proteins are interconnected with PrP<sup>C</sup>, and therefore, the role of PrP in the diseases linked to them are discussed.

### PrP<sup>C</sup> and Tauopathies

Tauopathies are a group of diseases that have in common the deposition of abnormal tau in the nervous system. They comprise AD, Pick's disease, progressive supranuclear palsy, corticobasal degeneration, and primary age-related tauopathy, among others (Kovacs, 2015). Normal Tau, which is a microtubule-associated protein, plays a role in the stabilization of neuronal microtubules. In pathological conditions, tau undergoes phosphorylation and forms aggregates that are neurotoxic (Avila et al., 2004).

Regarding its relation to PrP<sup>C</sup>, *in vitro* and *in vivo* studies have found an association between PrP<sup>C</sup> and hyperphosphorylated tau forms, particularly with tau N-terminal region (De Cecco et al., 2020; Legname and Scialo, 2020). Electrophysiological experiments showed that antibodies against PrP<sup>C</sup> (6D11, MI-0131) could prevent LTP impairment induced by tau toxicity (Ondrejcek et al., 2018). At present, it has been reported that other antibodies against different epitopes of PrP<sup>C</sup> (POM 3, 4, 12) are able to impair the uptake of tau amyloid fibrils in mouse neuroblastoma cells (De Cecco et al., 2020). In contrast, it has been described that tau is a transcription regulator for *PRPN* gene in AD models (Lidon et al., 2020), linking both proteins in the progression of tauopathies.

### PrP<sup>C</sup> and $\alpha$ -Synuclein

The misfolding and accumulation of  $\alpha$ -synuclein is involved in a group of pathologies known as synucleinopathies, such as PD, dementia with Lewy bodies (LBD), and multiple system atrophy (MSA). For instance, histopathological biomarker detected in patients with PD has been classically associated with abnormal deposits of  $\alpha$ -syn, which mainly affects nigral dopaminergic system at the intracellular level, also called Lewy bodies (Kalia and Lang, 2015).

In these diseases, similar to other proteinopathies, fibrillar forms of  $\alpha$ -syn spread from one cell to another. One of the mechanisms that this form of  $\alpha$ -syn uses to enter cell is clathrin-dependent endocytosis, a process that requires the interaction with the TM protein lymphocyte-activation gene 3 (*LAG3*) (De Cecco and Legname, 2018). Other protein that was reported to be involved in the internalization of  $\alpha$ -syn is PrP<sup>C</sup> (Aulic et al., 2017). Cells that express PrP<sup>C</sup> are able to internalize more amyloid  $\alpha$ -syn fibrils compared to cells that do not express it; therefore, PrP<sup>C</sup> favors cell-to-cell transmission (Aulic et al., 2017). In contrast, when these cells are infected with prions,  $\alpha$ -syn reduces prion replication, especially due to PrP<sup>C</sup>  $\alpha$  cleavage, producing C1 and N1 that are neuroprotectors (Aulic et al., 2017).

Further analyses agreed on the connection between PrP<sup>C</sup> and  $\alpha$ -syn: overexpression of PrP<sup>C</sup> in the striatum potentiates neurodegeneration, thereby altering  $\alpha$ -syn propagation and toxicity. Electrophysiological and molecular approaches showed that antibodies against PrP<sup>C</sup> 6D11 could abolish LTP impairment, calcium dyshomeostasis, and cell degeneration induced by  $\alpha$ -syn toxicity (Ferreira et al., 2017; Legname and Scialo, 2020).

## PrP<sup>C</sup> and TDP-43

Frontotemporal lobar degeneration (FTLD), a neurodegenerative syndrome in frontal and anterior temporal lobes (Rabinovici and Miller, 2010), and ALS, a motor neuron disorder characterized by degeneration in the upper and lower motor neurons (Prasad et al., 2019), are two distinct diseases that shared a histopathological hallmark: inclusion bodies composed of cytoplasmic deposits of the nuclear TDP-43 protein (Scialo et al., 2021). Under physiological conditions, TDP-43 is a transcriptional repressor that binds to chromosomally integrated TAR DNA. Nevertheless, a hyper-phosphorylated, ubiquitinated, and cleaved form of TDP-43 (pathological TDP-43) is the major disease protein in ubiquitin-positive, tau-, and  $\alpha$ -synuclein-negative FTLD and in ALS (Mackenzie et al., 2011; Brauer et al., 2018).

It was observed *in vitro* that TDP-43 fibrils bind to recombinant PrP<sup>C</sup>. Also, *in vitro*, it was shown that full-length mouse (Mu)PrP<sup>C</sup> as well as human (Hu)PrP<sup>C</sup> act as a membrane receptor of TDP-43 in its fibrillar conformation, inducing the formation of intracytoplasmic aggregates and cell death (Scialo et al., 2021). In addition, the overexpression of PrP<sup>C</sup> in human and mouse cell lines was directly correlated with the internalization of TDP-43 fibrils. Increased internalization was associated with detrimental consequences in all PrP-overexpressing cell lines (Scialo et al., 2021).

As for other amyloids, treatment with TDP-43 fibrils induced a reduction in the accumulation of the misfolded form of PrP<sup>C</sup>, PrP<sup>Sc</sup>, in cells chronically infected with prions. Our results expand the list of misfolded proteins whose uptake and detrimental effects are mediated by PrP<sup>C</sup>, which encompass almost all pathological amyloids involved in neurodegeneration (Scialo et al., 2021).

## PrP<sup>C</sup> IN AGING AND OTHER ABNORMAL PROCESSES

As we mentioned earlier, PrP<sup>C</sup> is mostly expressed in the brain. It is especially expressed in the hippocampus and it increases in the aging brain (Williams et al., 2004; Benvegnu et al., 2010). Aging, being the main risk factor for NDs (Wyss-Coray, 2016; Hou et al., 2019), can lead to cognitive impairment, affecting information processing and memory (Hedden and Gabrieli, 2004). Since PrP<sup>C</sup> has shown to participate in neuroprotection, metal homeostasis, and most probably as an antioxidant, it has been suggested that it may play a role in aging (Gasperini and Legname, 2014). In fact, in prion diseases, the function of PrP<sup>C</sup> is lost due to conversion into PrP<sup>Sc</sup> and this event could also be related to the progression of the disease (Gasperini and Legname, 2014). Furthermore, the biochemical properties of PrP<sup>C</sup> are altered during aging (Gasperini and Legname, 2014). Even though it is likely that PrP<sup>C</sup> is involved in behavior and learning processes during aging, the analyses performed so far in PrP<sup>C</sup> KO mice are not conclusive, probably due to differences in mouse models and age (Gasperini and Legname, 2014). Zurich old KO mice exhibit alteration in nest building behavior and decline in associative learning

compared to wild-type mice. At molecular level, mice lacking PrP<sup>C</sup> showed alterations in cytoskeletal proteins, due to the lower phosphorylation of the neurofilament heavy chain and reduction in B-tubulin III-positive neurons in the hippocampus (Gasperini and Legname, 2014; Schmitz et al., 2014). This might be related to neuronal structure changes due to the absence of PrP<sup>C</sup> and therefore a cellular explanation to behavioral abnormalities (Gasperini and Legname, 2014; Schmitz et al., 2014). Even though most studies suggest a role for PrP<sup>C</sup> in aging, more are still needed to better define this role.

## CONCLUSION

Although PrP<sup>C</sup> studies started from a pathological context, such as prion diseases, in recent years, studies of its functions in physiological terms increased, especially in the nervous system where this protein participates in relevant functions in neural networks, from neurite growth to ion channel association. Despite its important role, it remains a challenge to determine why the lack PrP<sup>C</sup> does not show a relevant phenotype and how other proteins might compensate the absence of PrP.

Recently, the role of PrP<sup>C</sup> in AD has emerged as crucial, supported by several studies. As this protein does not present TM spans, its interaction with other TM proteins must be key for its role in mediating physiological and pathological phenomena. Since Fyn kinase is a protein involved in both physiological and pathological PrP<sup>C</sup>-mediated responses, more studies are needed to understand the differences in the signaling in both processes.

With the discovery that PrP<sup>C</sup> is the main receptor for A $\beta$ O<sub>2</sub>, more studies are needed to determine whether PrP or other proteins in the pathological pathway might be a target for AD therapy and other NDs.

## AUTHOR CONTRIBUTIONS

JDP and PS contributed equally to this work in the information search and in the preparation of the manuscript. CMM, JDP, PS, BP, KE, and MEC participated in the figure designed and information search. GMC, JF, HR, CIR, and CMM conducted the manuscript preparation and edited the text.

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## REFERENCES

- Acevedo-Morantes, C. Y., and Wille, H. (2014). The structure of human prions: from biology to structural models-considerations and pitfalls. *Viruses* 6, 3875–3892. doi: 10.3390/v6103875
- Alper, T., Cramp, W. A., Haig, D. A., and Clarke, M. C. (1967). Does the agent of scrapie replicate without nucleic acid? *Nature* 214, 764–766. doi: 10.1038/214764a0
- Alvarez-Laviada, A., Kadurin, I., Senatore, A., Chiesa, R., and Dolphin, A. C. (2014). The inhibition of functional expression of calcium channels by prion protein demonstrates competition with alpha2delta for GPI-anchoring pathways. *Biochem. J.* 458, 365–374. doi: 10.1042/BJ20131405
- Aulic, S., Masperone, L., Narkiewicz, J., Isopi, E., Bistaffa, E., Ambrosetti, E., et al. (2017). Alpha-Synuclein amyloids hijack prion protein to gain cell entry, facilitate cell-to-cell spreading and block prion replication. *Sci. Rep.* 7:10050. doi: 10.1038/s41598-017-10236-x
- Avila, J., Lucas, J. J., Perez, M., and Hernandez, F. (2004). Role of tau protein in both physiological and pathological conditions. *Physiol. Rev.* 84, 361–384.
- Barry, A. E., Klyubin, I., Mc Donald, J.M., Mably, A. J., Farrell, M. A., Scott, M., et al. (2011). Alzheimer's disease brain-derived amyloid-beta-mediated inhibition of LTP in vivo is prevented by immunotargeting cellular prion protein. *J. Neurosci.* 31, 7259–7263. doi: 10.1523/JNEUROSCI.6500-10.2011
- Beland, M., and Roucou, X. (2012). The prion protein unstructured N-terminal region is a broad-spectrum molecular sensor with diverse and contrasting potential functions. *J. Neurochem.* 120, 853–868. doi: 10.1111/j.1471-4159.2011.07613.x
- Benvegnu, S., Poggolini, I., and Legname, G. (2010). Neurodevelopmental expression and localization of the cellular prion protein in the central nervous system of the mouse. *J. Comp. Neurol.* 518, 1879–1891. doi: 10.1002/cne.22357
- Beraldo, F. H., Arantes, C. P., Santos, T. G., Queiroz, N. G., Young, K., Rylett, R. J., et al. (2010). Role of alpha7 nicotinic acetylcholine receptor in calcium signaling induced by prion protein interaction with stress-inducible protein 1. *J. Biol. Chem.* 285, 36542–36550. doi: 10.1074/jbc.M110.157263
- Beringue, V., Mallinson, G., Kaiser, M., Tayebi, M., Sattar, Z., Jackson, G., et al. (2003). Regional heterogeneity of cellular prion protein isoforms in the mouse brain. *Brain* 126, 2065–2073.
- Biasini, E., Turnbaugh, J. A., Unterberger, U., and Harris, D. A. (2012). Prion protein at the crossroads of physiology and disease. *Trends Neurosci.* 35, 92–103.
- Black, S. A., Stys, P. K., Zamponi, G. W., and Tsutsui, S. (2014). Cellular prion protein and NMDA receptor modulation: protecting against excitotoxicity. *Front. Cell Dev. Biol.* 2:45. doi: 10.3389/fcell.2014.00045
- Bolton, D. C., McKinley, M. P., and Prusiner, S. B. (1982). Identification of a protein that purifies with the scrapie prion. *Science* 218, 1309–1311. doi: 10.1126/science.6815801
- Botto, L., Cunati, D., Coco, S., Sesana, S., Bulbarelli, A., Biasini, E., et al. (2014). Role of lipid rafts and GM1 in the segregation and processing of prion protein. *PLoS One* 9:e98344. doi: 10.1371/journal.pone.0098344
- Brauer, S., Zimyanin, V., and Hermann, A. (2018). Prion-like properties of disease-relevant proteins in amyotrophic lateral sclerosis. *J. Neural. Transm. (Vienna)* 125, 591–613. doi: 10.1007/s00702-018-1851-y
- Bueler, H., Fischer, M., Lang, Y., Bluethmann, H., Lipp, H. P., DeArmond, S. J., et al. (1992). Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* 356, 577–582. doi: 10.1038/356577a0
- Campbell, L., Gill, A. C., McGovern, G., Jalland, C. M., Hopkins, J., Tranulis, M. A., et al. (2013). The PrP(C) C1 fragment derived from the ovine A136R154R171PRNP allele is highly abundant in sheep brain and inhibits fibrillisation of full-length PrP(C) protein in vitro. *Biochim. Biophys. Acta* 1832, 826–836. doi: 10.1016/j.bbdis.2013.02.020
- Campiglio, M., and Flucher, B. E. (2015). The role of auxiliary subunits for the functional diversity of voltage-gated calcium channels. *J. Cell Physiol.* 230, 2019–2031. doi: 10.1002/jcp.24998
- Carulla, P., Bribian, A., Rangel, A., Gavin, R., Ferrer, I., Caelles, C., et al. (2011). Neuroprotective role of PrP<sup>C</sup> against kainate-induced epileptic seizures and cell death depends on the modulation of JNK3 activation by GluR6/7-PSD-95 binding. *Mol. Biol. Cell* 22, 3041–3054. doi: 10.1091/mbc.E11-04-0321
- Carulla, P., Llorens, F., Matamoros-Angles, A., Aguilar-Calvo, P., Espinosa, J. C., Gavin, R., et al. (2015). Involvement of PrP(C) in kainate-induced excitotoxicity in several mouse strains. *Sci. Rep.* 5:11971. doi: 10.1038/srep11971
- Castle, A. R., and Gill, A. C. (2017). Physiological functions of the cellular prion protein. *Front. Mol. Biosci.* 4:19. doi: 10.3389/fmolb.2017.00019
- Chakrabarti, O., Ashok, A., and Hegde, R. S. (2009). Prion protein biosynthesis and its emerging role in neurodegeneration. *Trends Biochem. Sci.* 34, 287–295. doi: 10.1016/j.tibs.2009.03.001
- Chater, T. E., and Goda, Y. (2014). The role of AMPA receptors in postsynaptic mechanisms of synaptic plasticity. *Front. Cell Neurosci.* 8:401. doi: 10.3389/fncel.2014.00401
- Chen, S., Yadav, S. P., and Surewicz, W. K. (2010). Interaction between human prion protein and amyloid-beta (Aβeta) oligomers: role of N-terminal residues. *J. Biol. Chem.* 285, 26377–26383. doi: 10.1074/jbc.M110.145516
- Chesebro, B., Race, R., Wehrly, K., Nishio, J., Bloom, M., Lechner, D., et al. (1985). Identification of scrapie prion protein-specific mRNA in scrapie-infected and uninfected brain. *Nature* 315, 331–333. doi: 10.1038/315331a0
- Choi, D. W. (1992). Excitotoxic cell death. *J. Neurobiol.* 23, 1261–1276.
- Ciric, D., and Rezaei, H. (2015). Biochemical insight into the prion protein family. *Front. Cell Dev. Biol.* 3:5. doi: 10.3389/fcell.2015.00005
- Cline, E. N., Bicca, M. A., Viola, K. L., and Klein, W. L. (2018). The Amyloid-beta oligomer hypothesis: beginning of the third decade. *J. Alzheimers Dis.* 64, S567–S610. doi: 10.3233/JAD-179941
- Colling, S. B., Collinge, J., and Jefferys, J. G. (1996). Hippocampal slices from prion protein null mice: disrupted Ca(2+)-activated K+ currents. *Neurosci. Lett.* 209, 49–52. doi: 10.1016/0304-3940(96)12596-9
- Collinge, J. (2001). Prion diseases of humans and animals: their causes and molecular basis. *Annu. Rev. Neurosci.* 24, 519–550.
- Collinge, J., Whittington, M. A., Sidle, K. C., Smith, C. J., Palmer, M. S., Clarke, A. R., et al. (1994). Prion protein is necessary for normal synaptic function. *Nature* 370, 295–297. doi: 10.1038/370295a0
- Corbett, G. T., Wang, Z., Hong, W., Colom-Cadena, M., Rose, J., Liao, M., et al. (2020). PrP is a central player in toxicity mediated by soluble aggregates of neurodegeneration-causing proteins. *Acta Neuropathol.* 139, 503–526. doi: 10.1007/s00401-019-02114-9
- Crozet, C., Beranger, F., and Lehmann, S. (2008). Cellular pathogenesis in prion diseases. *Vet. Res.* 39:44. doi: 10.1051/vetres:2008021
- De Cecco, E., Celauro, L., Vanni, S., Grandolfo, M., Bistaffa, E., Moda, F., et al. (2020). The uptake of tau amyloid fibrils is facilitated by the cellular prion protein and hampers prion propagation in cultured cells. *J. Neurochem.* 155, 577–591. doi: 10.1111/jnc.15040
- De Cecco, E., and Legname, G. (2018). The role of the prion protein in the internalization of alpha-synuclein amyloids. *Prion* 12, 23–27. doi: 10.1080/19336896.2017.1423186
- Dohler, F., Sepulveda-Falla, D., Krasemann, S., Altmeyen, H., Schluter, H., Hildebrand, D., et al. (2014). High molecular mass assemblies of amyloid-β oligomers bind prion protein in patients with Alzheimer's disease. *Brain* 137, 873–886. doi: 10.1093/brain/awt375
- Dron, M., Moudjou, M., Chapuis, J., Salamat, M. K., Bernard, J., Cronier, S., et al. (2010). Endogenous proteolytic cleavage of disease-associated prion protein to produce C2 fragments is strongly cell- and tissue-dependent. *J. Biol. Chem.* 285, 10252–10264. doi: 10.1074/jbc.M109.083857
- Engelke, A. D., Gonsberg, A., Thapa, S., Jung, S., Ulbrich, S., Seidel, R., et al. (2018). Dimerization of the cellular prion protein inhibits propagation of scrapie prions. *J. Biol. Chem.* 293, 8020–8031. doi: 10.1074/jbc.RA117.000990
- Fabiani, C., and Antolini, S. S. (2019). Alzheimer's disease as a membrane disorder: spatial cross-talk among beta-amyloid peptides, nicotinic acetylcholine receptors and lipid rafts. *Front. Cell Neurosci.* 13:309. doi: 10.3389/fncel.2019.00309
- Ferreira, D. G., Temido-Ferreira, M., Vicente Miranda, H., Batalha, V. L., Coelho, J. E., and Szego, E. M. I., et al. (2017). alpha-synuclein interacts with PrP(C) to induce cognitive impairment through mGluR5 and NMDAR2B. *Nat. Neurosci.* 20, 1569–1579. doi: 10.1038/nn.4648
- Fluharty, B. R., Biasini, E., Stravalaci, M., Sclip, A., Diomedea, L., Balducci, C., et al. (2013). An N-terminal fragment of the prion protein binds to amyloid-beta oligomers and inhibits their neurotoxicity in vivo. *J. Biol. Chem.* 288, 7857–7866. doi: 10.1074/jbc.M112.423954

- Gasparini, L., and Legname, G. (2014). Prion protein and aging. *Front. Cell Dev. Biol.* 2:44. doi: 10.3389/fcell.2014.00044
- Gasparini, L., Meneghetti, E., Pastore, B., Benetti, F., and Legname, G. (2015). Prion protein and copper cooperatively protect neurons by modulating NMDA receptor through S-nitrosylation. *Antioxid. Redox Signal.* 22, 772–784. doi: 10.1089/ars.2014.6032
- Gavin, R., Lidon, L., Ferrer, I., and Del Rio, J. A. (2020). The quest for cellular prion protein functions in the aged and neurodegenerating brain. *Cells* 9:591. doi: 10.3390/cells9030591
- Ghirardini, E., Restelli, E., Morini, R., Bertani, I., Ortolan, D., Perrucci, F., et al. (2020). Mutant prion proteins increase calcium permeability of AMPA receptors, exacerbating excitotoxicity. *PLoS Pathog.* 16:e1008654. doi: 10.1371/journal.ppat.1008654
- Giachin, G., Mai, P. T., Tran, T. H., Salzano, G., Benetti, F., Migliorati, V., et al. (2015). The non-octarepeat copper binding site of the prion protein is a key regulator of prion conversion. *Sci. Rep.* 5:15253. doi: 10.1038/srep15253
- Gimbel, D. A., Nygaard, H. B., Coffey, E. E., Gunther, E. C., Lauren, J., Gimbel, Z. A., et al. (2010). Memory impairment in transgenic Alzheimer mice requires cellular prion protein. *J. Neurosci.* 30, 6367–6374. doi: 10.1523/JNEUROSCI.0395-10.2010
- Griffith, J. S. (1967). Self-replication and scrapie. *Nature* 215, 1043–1044. doi: 10.1038/2151043a0
- Hedden, T., and Gabrieli, J. D. (2004). Insights into the ageing mind: a view from cognitive neuroscience. *Nat. Rev. Neurosci.* 5, 87–96. doi: 10.1038/nrn1323
- Hegde, R. S., Mastrianni, J. A., Scott, M. R., DeFea, K. A., Tremblay, P., Torchia, M., et al. (1998). A transmembrane form of the prion protein in neurodegenerative disease. *Science* 279, 827–834. doi: 10.1126/science.279.5352.827
- Heller, U., Winklhofer, K. F., Heske, J., Reintjes, A., and Tatzelt, J. (2003). Post-translational import of the prion protein into the endoplasmic reticulum interferes with cell viability: a critical role for the putative transmembrane domain. *J. Biol. Chem.* 278, 36139–36147. doi: 10.1074/jbc.M304002200
- Hermes, J. W., Korte, S., Gall, S., Schneider, I., Dunker, S., and Kretzschmar, H. A. (2000). Altered intracellular calcium homeostasis in cerebellar granule cells of prion protein-deficient mice. *J. Neurochem.* 75, 1487–1492. doi: 10.1046/j.1471-4159.2000.0751487.x
- Hermes, J. W., Tings, T., Dunker, S., and Kretzschmar, H. A. (2001). Prion protein affects Ca<sup>2+</sup>-activated K<sup>+</sup> currents in cerebellar purkinje cells. *Neurobiol. Dis.* 8, 324–330. doi: 10.1006/mbdi.2000.0369
- Heske, J., Heller, U., Winklhofer, K. F., and Tatzelt, J. (2004). The C-terminal globular domain of the prion protein is necessary and sufficient for import into the endoplasmic reticulum. *J. Biol. Chem.* 279, 5435–5443. doi: 10.1074/jbc.M309570200
- Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S. G., Croteau, D. L., et al. (2019). Ageing as a risk factor for neurodegenerative disease. *Nat. Rev. Neurol.* 15, 565–581.
- Huang, S., Chen, L., Bladen, C., Stys, P. K., and Zamponi, G. W. (2018). Differential modulation of NMDA and AMPA receptors by cellular prion protein and copper ions. *Mol. Brain* 11:62. doi: 10.1186/s13041-018-0406-3
- Isaacs, J. D., Jackson, G. S., and Altmann, D. M. (2006). The role of the cellular prion protein in the immune system. *Clin. Exp. Immunol.* 146, 1–8.
- Jeon, J. W., Park, B. C., Jung, J. G., Jang, Y. S., Shin, E. C., and Park, Y. W. (2013). The Soluble form of the cellular prion protein enhances phagocytic activity and cytokine production by human monocytes via activation of ERK and NF-kappaB. *Immune Netw.* 13, 148–156. doi: 10.4110/in.2013.13.4.148
- Jeong, J. K., and Park, S. Y. (2015). Neuroprotective effect of cellular prion protein (PrP<sup>C</sup>) is related with activation of alpha7 nicotinic acetylcholine receptor (alpha7nAChR)-mediated autophagy flux. *Oncotarget* 6, 24660–24674. doi: 10.18632/oncotarget.4953
- Kalia, L. V., and Lang, A. E. (2015). Parkinson's disease. *Lancet* 386, 896–912.
- Kang, M., Kim, S. Y., An, S. S., and Ju, Y. R. (2013). Characterizing affinity epitopes between prion protein and beta-amyloid using an epitope mapping immunoassay. *Exp. Mol. Med.* 45:e34. doi: 10.1038/emmm.2013.63
- Kellett, K. A., and Hooper, N. M. (2009). Prion protein and Alzheimer disease. *Prion* 3, 190–194. doi: 10.4161/pri.3.4.9980
- Khosravani, H., Zhang, Y., and Zamponi, G. W. (2008b). Cellular prion protein null mice display normal AMPA receptor mediated long term depression. *Prion* 2, 48–50. doi: 10.4161/pri.2.2.6628
- Khosravani, H., Zhang, Y., Tsutsui, S., Hameed, S., Altier, C., Hamid, J., et al. (2008a). Prion protein attenuates excitotoxicity by inhibiting NMDA receptors. *J. Cell Biol.* 181, 551–65.
- Kim, J., Nadal, M. S., Clemens, A. M., Baron, M., Jung, S. C., Misumi, Y., et al. (2008). Kv4 accessory protein DPPX (DPP6) is a critical regulator of membrane excitability in hippocampal CA1 pyramidal neurons. *J. Neurophysiol.* 100, 1835–1847. doi: 10.1152/jn.90261.2008
- Kim, S. J., Rahbar, R., and Hegde, R. S. (2001). Combinatorial control of prion protein biogenesis by the signal sequence and transmembrane domain. *J. Biol. Chem.* 276, 26132–26140. doi: 10.1074/jbc.M101638200
- Kleene, R., Loers, G., Langer, J., Frobert, Y., Buck, F., and Schachner, M. (2007). Prion protein regulates glutamate-dependent lactate transport of astrocytes. *J. Neurosci.* 27, 12331–12340. doi: 10.1523/JNEUROSCI.1358-07.2007
- Kostylev, M. A., Kaufman, A. C., Nygaard, H. B., Patel, P., Haas, L. T., Gunther, E. C., et al. (2015). Prion-protein-interacting amyloid-beta oligomers of high molecular weight are tightly correlated with memory impairment in multiple alzheimer mouse models. *J. Biol. Chem.* 290, 17415–17438. doi: 10.1074/jbc.M115.643577
- Kovacs, G. G. (2015). Invited review: neuropathology of tauopathies: principles and practice. *Neuropathol. Appl. Neurobiol.* 41, 3–23. doi: 10.1111/nan.12208
- Kuffer, A., Lakkaraju, A. K., Mogha, A., Petersen, S. C., Airich, K., Doucerain, C., et al. (2016). The prion protein is an agonistic ligand of the G protein-coupled receptor Adrg6. *Nature* 536, 464–468. doi: 10.1038/nature19312
- Lambert, M. P., Barlow, A. K., Chromy, B. A., Edwards, C., Freed, R., Liosatos, M., et al. (1998). Diffusible, nonfibrillar ligands derived from Abeta1-42 are potent central nervous system neurotoxins. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6448–6453. doi: 10.1073/pnas.95.11.6448
- Lansbury, P. T. (1994). Mechanism of scrapie replication. *Science* 265:1510. doi: 10.1126/science.8079159
- Larson, M., Sherman, M. A., Amar, F., Nuvolone, M., Schneider, J. A., Bennett, D. A., et al. (2012). The complex PrP(c)-Fyn couples human oligomeric Abeta with pathological tau changes in Alzheimer's disease. *J. Neurosci.* 32, 16857–16871. doi: 10.1523/JNEUROSCI.1858-12.2012
- Lau, A., McDonald, A., Daude, N., Mays, C. E., Walter, E. D., Aglietti, R., et al. (2015). Octarepeat region flexibility impacts prion function, endoproteolysis and disease manifestation. *EMBO Mol. Med.* 7, 339–356. doi: 10.15252/emmm.201404588
- Laurén, J., Gimbel, D. A., Nygaard, H. B., Gilbert, J. W., and Strittmatter, S. M. (2009). Cellular prion protein mediates impairment of synaptic plasticity by amyloid-beta oligomers. *Nature* 457, 1128–1132. doi: 10.1038/nature07761
- Legname, G. (2017). Elucidating the function of the prion protein. *PLoS Pathog.* 13:e1006458. doi: 10.1371/journal.ppat.1006458
- Legname, G., and Scialo, C. (2020). On the role of the cellular prion protein in the uptake and signaling of pathological aggregates in neurodegenerative diseases. *Prion* 14, 257–270.
- Liang, J., and Kong, Q. (2012). alpha-Cleavage of cellular prion protein. *Prion* 6, 453–460.
- Lidon, L., Vergara, C., Ferrer, I., Hernandez, F., Avila, J., Del Rio, J. A., et al. (2020). Tau protein as a new regulator of cellular prion protein transcription. *Mol. Neurobiol.* 57, 4170–4186.
- Lima, F. R., Arantes, C. P., Muras, A. G., Nomizo, R., Brentani, R. R., and Martins, V. R. (2007). Cellular prion protein expression in astrocytes modulates neuronal survival and differentiation. *J. Neurochem.* 103, 2164–2176.
- Linden, R. (2017). The biological function of the prion protein: a cell surface scaffold of signaling modules. *Front. Mol. Neurosci.* 10:77. doi: 10.3389/fnmol.2017.00077
- Linden, R., Martins, V. R., Prado, M. A., Cammarota, M., Izquierdo, I., and Brentani, R. R. (2008). Physiology of the prion protein. *Physiol. Rev.* 88, 673–728.
- Llinas, R., Steinberg, I. Z., and Walton, K. (1976). Presynaptic calcium currents and their relation to synaptic transmission: voltage clamp study in squid giant synapse and theoretical model for the calcium gate. *Proc. Natl. Acad. Sci. U. S. A.* 73, 2918–2922. doi: 10.1073/pnas.73.8.2918
- Llorens, F., Carulla, P., Villa, A., Torres, J. M., Fortes, P., Ferrer, I., et al. (2013). PrP(C) regulates epidermal growth factor receptor function and cell shape dynamics in Neuro2a cells. *J. Neurochem.* 127, 124–138. doi: 10.1111/jnc.12283

- Mackenzie, I. R., Neumann, M., Baborie, A., Sampathu, D. M., Du Plessis, D., Jaros, E., et al. (2011). A harmonized classification system for FTLD-TDP pathology. *Acta Neuropathol.* 122, 111–113. doi: 10.1007/s00401-011-0845-8
- Maglio, L. E., Perez, M. F., Martins, V. R., Brentani, R. R., and Ramirez, O. A. (2004). Hippocampal synaptic plasticity in mice devoid of cellular prion protein. *Brain Res. Mol. Brain Res.* 131, 58–64.
- Malaga-Trillo, E., and Ochs, K. (2016). Uncontrolled SFK-mediated protein trafficking in prion and Alzheimer's disease. *Prion* 10, 352–361. doi: 10.1080/19336896.2016.1221873
- Mallucci, G. R., Ratte, S., Asante, E. A., Linehan, J., Gowland, I., Jefferys, J. G., et al. (2002). Post-natal knockout of prion protein alters hippocampal CA1 properties, but does not result in neurodegeneration. *EMBO J.* 21, 202–210. doi: 10.1093/emboj/21.3.202
- Mange, A., Beranger, F., Peoc'h, K., Onodera, T., Frobert, Y., and Lehmann, S. (2004). Alpha- and beta- cleavages of the amino-terminus of the cellular prion protein. *Biol. Cell* 96, 125–132. doi: 10.1016/j.biocel.2003.11.007
- Manson, J., West, J. D., Thomson, V., McBride, P., Kaufman, M. H., and Hope, J. (1992). The prion protein gene: a role in mouse embryogenesis? *Development* 115, 117–122.
- Manson, J. C., Clarke, A. R., Hooper, M. L., Aitchison, L., McConnell, I., and Hope, J. (1994). 129/Ola mice carrying a null mutation in PrP that abolishes mRNA production are developmentally normal. *Mol. Neurobiol.* 8, 121–127. doi: 10.1007/BF02780662
- Marijanovic, Z., Caputo, A., Campana, V., and Zurzolo, C. (2009). Identification of an intracellular site of prion conversion. *PLoS Pathog.* 5:e1000426. doi: 10.1371/journal.ppat.1000426
- Martellucci, S., Santacroce, C., Santilli, F., Manganelli, V., Sorice, M., and Mattei, V. (2020). Prion protein in stem cells: a lipid raft component involved in the cellular differentiation process. *Int. J. Mol. Sci.* 21:4168. doi: 10.3390/ijms21114168
- Martinez, J., Sanchez, R., Castellanos, M., Makarava, N., Aguzzi, A., Baskakov, I. V., et al. (2015). PrP charge structure encodes interdomain interactions. *Sci. Rep.* 5:13623. doi: 10.1038/srep13623
- Mercer, R. C., Ma, L., Watts, J. C., Strome, R., Wohlgemuth, S., Yang, J., et al. (2013). The prion protein modulates A-type K<sup>+</sup> currents mediated by Kv4.2 complexes through dipeptidyl aminopeptidase-like protein 6. *J. Biol. Chem.* 288, 37241–37255. doi: 10.1074/jbc.M113.488650
- Miranzadeh Mahabadi, H., and Taghibiglou, C. (2020). Cellular Prion Protein (PrP<sup>C</sup>): putative interacting partners and consequences of the interaction. *Int. J. Mol. Sci.* 21:7058.
- Munoz-Montesino, C., Larkem, D., Barbereau, C., Igel-Egalon, A., Truchet, S., Jacquet, E., et al. (2020). A seven-residue deletion in PrP leads to generation of a spontaneous prion formed from C-terminal C1 fragment of PrP. *J. Biol. Chem.* 295, 14025–14039. doi: 10.1074/jbc.RA120.014738
- Munoz-Montesino, C., Sizun, C., Moudjou, M., Herzog, L., Reine, F., Chapuis, J., et al. (2016). Generating bona fide mammalian prions with internal deletions. *J. Virol.* 90, 6963–6975. doi: 10.1128/JVI.00555-16
- Munoz-Montesino, C., Sizun, C., Moudjou, M., Herzog, L., Reine, F., Igel-Egalon, A., et al. (2017). A stretch of residues within the protease-resistant core is not necessary for prion structure and infectivity. *Prion* 11, 25–30. doi: 10.1080/19336896.2016.1274851
- Musiek, E. S., and Schindler, S. E. (2013). Alzheimer disease: current concepts & future directions. *Mo. Med.* 110, 395–400.
- Nicoll, A. J., Panico, S., Freir, D. B., Wright, D., Terry, C., Risse, E., et al. (2013). Amyloid- $\beta$  nanotubes are associated with prion protein-independent synaptotoxicity. *Nat. Commun.* 4:2416. doi: 10.1038/ncomms3416
- Nguyen, X. T. A., Tran, T. H., Cojoc, D., and Legname, G. (2019). Copper binding regulates cellular prion protein function. *Mol. Neurobiol.* 56, 6121–6133. doi: 10.1007/s12035-019-1510-9
- Ondrejcek, T., Klyubin, I., Corbett, G. T., Fraser, G., Hong, W., Mably, A. J., et al. (2018). Cellular prion protein mediates the disruption of hippocampal synaptic plasticity by soluble tau in vivo. *J. Neurosci.* 38, 10595–10606. doi: 10.1523/JNEUROSCI.1700-18.2018
- Pantera, B., Bini, C., Cirri, P., Paoli, P., Camici, G., Manao, G., et al. (2009). PrP<sup>C</sup> activation induces neurite outgrowth and differentiation in PC12 cells: role for caveolin-1 in the signal transduction pathway. *J. Neurochem.* 110, 194–207. doi: 10.1111/j.1471-4159.2009.06123.x
- Park, J. Y., Jeong, J. K., Lee, J. H., Moon, J. H., Kim, S. W., Lee, Y. J., et al. (2015). Induction of cellular prion protein (PrP<sup>C</sup>) under hypoxia inhibits apoptosis caused by TRAIL treatment. *Oncotarget* 6, 5342–5353. doi: 10.18632/oncotarget.3028
- Peters, C., Espinoza, M. P., Gallegos, S., Opazo, C., and Aguayo, L. G. (2015). Alzheimer's Abeta interacts with cellular prion protein inducing neuronal membrane damage and synaptotoxicity. *Neurobiol. Aging* 36, 1369–1377. doi: 10.1016/j.neurobiolaging.2014.11.019
- Petit, C. S., Besnier, L., Morel, E., Rousset, M., and Thenet, S. (2013). Roles of the cellular prion protein in the regulation of cell-cell junctions and barrier function. *Tissue Barriers* 1:e24377. doi: 10.4161/tisb.24377
- Prado, M. B., Melo Escobar, M. I., Alves, R. N., Coelho, B. P., Fernandes, C. F. L., Boccacino, J. M., et al. (2020). Prion protein at the leading edge: its role in cell motility. *Int. J. Mol. Sci.* 21:6677. doi: 10.3390/ijms21186677
- Prasad, A., Bharathi, V., Sivalingam, V., Girdhar, A., and Patel, B. K. (2019). Molecular mechanisms of TDP-43 Misfolding and pathology in amyotrophic lateral sclerosis. *Front. Mol. Neurosci.* 12:25. doi: 10.3389/fnmol.2019.00025
- Premzl, M., and Gamulin, V. (2007). Comparative genomic analysis of prion genes. *BMC Genomics* 8:1. doi: 10.1186/1471-2164-8-1
- Prusiner, S. B. (1982). Novel proteinaceous infectious particles cause scrapie. *Science* 216, 136–144. doi: 10.1126/science.6801762
- Prusiner, S. B. (2012). Cell biology. A unifying role for prions in neurodegenerative diseases. *Science* 336, 1511–1513. doi: 10.1126/science.1222951
- Puzzo, D., Piacentini, R., Fa, M., Gulisano, W., Li Puma, D. D., Staniszewski, A., et al. (2017). LTP and memory impairment caused by extracellular Abeta and Tau oligomers is APP-dependent. *Elife* 6:e26991. doi: 10.7554/eLife.26991
- Rabinovici, G. D., and Miller, B. L. (2010). Frontotemporal lobar degeneration: epidemiology, pathophysiology, diagnosis and management. *CNS Drugs* 24, 375–398. doi: 10.2165/11533100-000000000-00000
- Rammes, G., Seeser, F., Mattusch, K., Zhu, K., Haas, L., Kummer, M., et al. (2018). The NMDA receptor antagonist Radiprodil reverses the synaptotoxic effects of different amyloid-beta (Abeta) species on long-term potentiation (LTP). *Neuropharmacology* 140, 184–192. doi: 10.1016/j.neuropharm.2018.07.021
- Rangel, A., Burgaya, F., Gavin, R., Soriano, E., Aguzzi, A., and Del Rio, J. A. (2007). Enhanced susceptibility of Prnp-deficient mice to kainate-induced seizures, neuronal apoptosis, and death: role of AMPA/kainate receptors. *J. Neurosci. Res.* 85, 2741–2755. doi: 10.1002/jnr.21215
- Riek, R., Hornemann, S., Wider, G., Billeter, M., Glockshuber, R., and Wuthrich, K. (1996). NMR structure of the mouse prion protein domain PrP(121–231). *Nature* 382, 180–182.
- Roffe, M., Beraldo, F. H., Bester, R., Nunziante, M., Bach, C., Mancini, G., et al. (2010). Prion protein interaction with stress-inducible protein 1 enhances neuronal protein synthesis via mTOR. *Proc. Natl. Acad. Sci. U. S. A.* 107, 13147–13152. doi: 10.1073/pnas.1000784107
- Rutishauser, D., Mertz, K. D., Moos, R., Brunner, E., Rulicke, T., Calella, A. M., et al. (2009). The comprehensive native interactor of a fully functional tagged prion protein. *PLoS One* 4:e4446. doi: 10.1371/journal.pone.0004446
- Sakaguchi, S., Katamine, S., Nishida, N., Moriuchi, R., Shigematsu, K., Sugimoto, T., et al. (1996). Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. *Nature* 380, 528–531. doi: 10.1038/380528a0
- Sakudo, A., Lee, D. C., Nakamura, I., Taniuchi, Y., Saeki, K., Matsumoto, Y., et al. (2005). Cell-autonomous PrP-Doppel interaction regulates apoptosis in PrP gene-deficient neuronal cells. *Biochem. Biophys. Res. Commun.* 333, 448–454. doi: 10.1016/j.bbrc.2005.05.128
- Salamat, K., Moudjou, M., Chapuis, J., Herzog, L., Jaumain, E., Beringue, V., et al. (2012). Integrity of helix 2-helix 3 domain of the PrP protein is not mandatory for prion replication. *J. Biol. Chem.* 287, 18953–18964. doi: 10.1074/jbc.M112.341677
- Salamat, M. K., Munoz-Montesino, C., Moudjou, M., Rezaei, H., Laude, H., Beringue, V., et al. (2013). Mammalian prions: tolerance to sequence changes-how far? *Prion* 7, 131–135. doi: 10.4161/pri.23110
- Salzano, G., Giachin, G., and Legname, G. (2019). Structural Consequences of Copper Binding to the Prion Protein. *Cells* 8:770. doi: 10.3390/cells8080770
- Santuccione, A., Sytnyk, V., Leshchyn'ska, I., and Schachner, M. (2005). Prion protein recruits its neuronal receptor NCAM to lipid rafts to activate p59fyn and to enhance neurite outgrowth. *J. Cell Biol.* 169, 341–354. doi: 10.1083/jcb.200409127

- Sarnataro, D., Pepe, A., and Zurzolo, C. (2017). Cell Biology of Prion Protein. *Prog. Mol. Biol. Transl. Sci.* 150, 57–82. doi: 10.1016/bs.pmbts.2017.06.018
- Schilling, K. M., Tao, L., Wu, B., Kiblen, J. T. M., Ubilla-Rodriguez, N. C., Pushie, M. J., et al. (2020). Both N-Terminal and C-Terminal histidine residues of the prion protein are essential for copper coordination and neuroprotective self-regulation. *J. Mol. Biol.* 432, 4408–4425. doi: 10.1016/j.jmb.2020.05.020
- Schmitt-Ulms, G., Hansen, K., Liu, J., Cowdrey, C., Yang, J., DeArmond, S. J., et al. (2004). Time-controlled transcardiac perfusion cross-linking for the study of protein interactions in complex tissues. *Nat. Biotechnol.* 22, 724–731. doi: 10.1038/nbt969
- Schmitt-Ulms, G., Legname, G., Baldwin, M. A., Ball, H. L., Bradon, N., Bosque, P. J., et al. (2001). Binding of neural cell adhesion molecules (N-CAMs) to the cellular prion protein. *J. Mol. Biol.* 314, 1209–1225. doi: 10.1006/jmbi.2000.5183
- Schmitz, M., Greis, C., Ottis, P., Silva, C. J., Schulz-Schaeffer, W. J., Wrede, A., et al. (2014). Loss of prion protein leads to age-dependent behavioral abnormalities and changes in cytoskeletal protein expression. *Mol. Neurobiol.* 50, 923–936. doi: 10.1007/s12035-014-8655-3
- Scialo, C., Celauro, L., Zattoni, M., Tran, T. H., Bistaffa, E., Moda, F., et al. (2021). The cellular prion protein increases the uptake and toxicity of TDP-43 Fibrils. *Viruses* 13:1625. doi: 10.3390/v13081625
- Selkoe, D. J. (2001). Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* 81, 741–766.
- Senatore, A., Colleoni, S., Verderio, C., Restelli, E., Morini, R., Condliffe, S. B., et al. (2012). Mutant PrP suppresses glutamatergic neurotransmission in cerebellar granule neurons by impairing membrane delivery of VGCC alpha(2)delta-1 Subunit. *Neuron* 74, 300–313. doi: 10.1016/j.neuron.2012.02.027
- Simons, K., and Gerl, M. J. (2010). Revitalizing membrane rafts: new tools and insights. *Nat. Rev. Mol. Cell Biol.* 11, 688–699. doi: 10.1038/nrm2977
- Smith, L. M., Kostylev, M. A., Lee, S., and Strittmatter, S. M. (2019). Systematic and standardized comparison of reported amyloid-beta receptors for sufficiency, affinity, and Alzheimer's disease relevance. *J. Biol. Chem.* 294, 6042–6053. doi: 10.1074/jbc.RA118.006252
- Smith, L. M., and Strittmatter, S. M. (2017). Binding sites for amyloid-beta oligomers and synaptic toxicity. *Cold Spring Harb. Perspect. Med.* 7:a024075. doi: 10.1101/cshperspect.a024075
- Sparkes, R. S., Simon, M., Cohn, V. H., Fournier, R. E., Lem, J., Klisak, I., et al. (1986). Assignment of the human and mouse prion protein genes to homologous chromosomes. *Proc. Natl. Acad. Sci. U. S. A.* 83, 7358–7362. doi: 10.1073/pnas.83.19.7358
- Stahl, N., Borchelt, D. R., Hsiao, K., and Prusiner, S. B. (1987). Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell* 51, 229–240. doi: 10.1016/0092-8674(87)90150-4
- Su, A. I., Wiltshire, T., Batalov, S., Lapp, H., Ching, K. A., Block, D., et al. (2004). A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc. Natl. Acad. Sci. U. S. A.* 101, 6062–6067. doi: 10.1073/pnas.0400782101
- Turnbaugh, J. A., Unterberger, U., Saa, P., Massignan, T., Fluharty, B. R., Bowman, F. P., et al. (2012). The N-terminal, polybasic region of PrP(C) dictates the efficiency of prion propagation by binding to PrP(Sc). *J. Neurosci.* 32, 8817–8830. doi: 10.1523/JNEUROSCI.1103-12.2012
- Urrea, L., Ferrer, I., Gavin, R., and Del Rio, J. A. (2017). The cellular prion protein (PrP(C)) as neuronal receptor for alpha-synuclein. *Prion* 11, 226–233. doi: 10.1080/19336896.2017.1334748
- Walsh, D. M., and Selkoe, D. J. (2007). A beta oligomers - a decade of discovery. *J. Neurochem.* 101, 1172–1184. doi: 10.1111/j.1471-4159.2006.04426.x
- Wang, F., Yin, S., Wang, X., Zha, L., Sy, M. S., and Ma, J. (2010). Role of the highly conserved middle region of prion protein (PrP) in PrP-lipid interaction. *Biochemistry* 49, 8169–8176. doi: 10.1021/bi101146v
- Warwicker, J. (2000). Modeling a prion protein dimer: predictions for fibril formation. *Biochem. Biophys. Res. Commun.* 278, 646–652. doi: 10.1006/bbrc.2000.3829
- Watt, N. T., Taylor, D. R., Kerrigan, T. L., Griffiths, H. H., Rushworth, J. V., Whitehouse, I. J., et al. (2012). Prion protein facilitates uptake of zinc into neuronal cells. *Nat. Commun.* 3:1134.
- Westergard, L., Christensen, H. M., and Harris, D. A. (2007). The cellular prion protein (PrP(C)): its physiological function and role in disease. *Biochim. Biophys. Acta* 1772, 629–644. doi: 10.1016/j.bbadis.2007.02.011
- Westergard, L., Turnbaugh, J. A., and Harris, D. A. (2011). A naturally occurring C-terminal fragment of the prion protein (PrP) delays disease and acts as a dominant-negative inhibitor of PrPSc formation. *J. Biol. Chem.* 286, 44234–44242. doi: 10.1074/jbc.M111.286195
- Wiatrak, B., Piasny, J., Kuzniarski, A., and Gasiorowski, K. (2021). Interactions of Amyloid-beta with Membrane Proteins. *Int. J. Mol. Sci.* 22:6075. doi: 10.3390/ijms22116075
- Williams, W. M., Stadtman, E. R., and Moskowitz, J. (2004). Ageing and exposure to oxidative stress in vivo differentially affect cellular levels of PrP in mouse cerebral microvessels and brain parenchyma. *Neuropathol. Appl. Neurobiol.* 30, 161–168. doi: 10.1111/j.1365-2990.2003.00523.x
- Wu, B., McDonald, A. J., Markham, K., Rich, C. B., McHugh, K. P., Tatzelt, J., et al. (2017). The N-terminus of the prion protein is a toxic effector regulated by the C-terminus. *Elife* 6:e23473. doi: 10.7554/eLife.23473
- Wulf, M. A., Senatore, A., and Aguzzi, A. (2017). The biological function of the cellular prion protein: an update. *BMC Biol.* 15:34. doi: 10.1186/s12915-017-0375-5
- Wyss-Coray, T. (2016). Ageing, neurodegeneration and brain rejuvenation. *Nature* 539, 180–186. doi: 10.1038/nature20411
- Yim, Y. I., Park, B. C., Yadavalli, R., Zhao, X., Eisenberg, E., and Greene, L. E. (2015). The multivesicular body is the major internal site of prion conversion. *J. Cell Sci.* 128, 1434–1443. doi: 10.1242/jcs.165472
- You, H., Tsutsui, S., Hameed, S., Kannanayakal, T. J., Chen, L., Xia, P., et al. (2012). Abeta neurotoxicity depends on interactions between copper ions, prion protein, and N-methyl-D-aspartate receptors. *Proc. Natl. Acad. Sci. U. S. A.* 109, 1737–1742. doi: 10.1073/pnas.1110789109
- Zanata, S. M., Lopes, M. H., Mercadante, A. F., Hajj, G. N., Chiarini, L. B., Nomizo, R., et al. (2002). Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. *EMBO J.* 21, 3307–3316. doi: 10.1093/emboj/cdf325
- Zeng, F., Watt, N. T., Walmsley, A. R., and Hooper, N. M. (2003). Tethering the N-terminus of the prion protein compromises the cellular response to oxidative stress. *J. Neurochem.* 84, 480–490. doi: 10.1046/j.1471-4159.2003.01529.x
- Zhang, Y., Zhao, Y., Zhang, L., Yu, W., Wang, Y., and Chang, W. (2019). Cellular prion protein as a receptor of Toxic Amyloid-beta42 oligomers is important for Alzheimer's disease. *Front. Cell Neurosci.* 13:339. doi: 10.3389/fncel.2019.00339
- Zuegg, J., and Gready, J. E. (2000). Molecular dynamics simulation of human prion protein including both N-linked oligosaccharides and the GPI anchor. *Glycobiology* 10, 959–974. doi: 10.1093/glycob/10.10.959

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